Vitamin B_{12}

Selected Annotated Bibliography

1954



MERCK & CO., INC

Manufacturing Chemists

RAHWAY NEW JERSEY

PRESENTED
by

Foreword

This Selected Annotated Bibliography on Vitamin B_{12} makes available the wealth of clinical and experimental data derived from the medical and related scientific literature from 1948 through the first half of 1953. Accordingly most of the abstracts originally published in the Annotated Bibliography on Vitamin B_{12} (1950) have been incorporated into this present and greatly expanded volume.

In Part I the material has been arranged with reference to therapeutic use of vitamin B_{12} in various systems or organs of the body and in certain physiologic and nutritional conditions and states—both from the clinical and the experimental standpoint. In Part II will be found analytical data pertaining to vitamin B_{12} itself, its identification, characteristics and properties, and other salient aspects.

It is hoped that this comprehensive collection of abstracts and reference data on vitamin B_{12} will prove useful to those engaged in basic and clinical research and in the practice of medicine.

MERCK & CO., INC.
CHEMICAL DIVISION
Rahway N J

MEDICAL DIVISION
January 1954

PRESEN[illegible]
by

PART I. THERAPEUTIC USES OF VITAMIN B_{12}

PART II. ANALYTICAL STUDIES OF VITAMIN B_{12}

Part I

Therapeutic Uses of Vitamin B_{12}

ANEMIAS

Macrocytic Anemia

1 STONE, R. E., and SPIES, T D *The effect of liver extract and vitamin B_{12} on the mucous membrane lesions of macrocytic anemia*, J Lab. & Clin. Med. 33 1019-1023, Aug. 1948.

In the authors experience, folic acid or thymine is without effect on very severe mucous membrane lesions of pernicious anemia, but liver extract produces prompt improvement which usually is maintained for one to nine months. Two patients who had a severe recurrence of such lesions have been treated with vitamin B_{12}, with at least temporary relief—they have been observed for two weeks to date. One case is reported in detail. In this case 15 mcg. was given intramuscularly Within 24 hours there were equivocal signs of improvement. After 48 hours hyperemia of the tongue and buccal mucosa had definitely decreased. By the fifth day the color of the mucosa of the tongue and oral cavity appeared nearly normal, the external fissures were dry and apparently healing, and the tongue was less swollen. By the thirteenth day many fine papillae were visible over the entire upper surface of the tongue, and the external fissures were completely healed. All soreness and burning sensation of the mouth, tongue, and external fissures began to subside within 48 hours after the injection and completely disappeared within five days.

2. PATEL, J C *Crystalline anti-pernicious-anaemia factor in treatment of two cases of tropical macrocytic anaemia*, Brit. M. J 2 934-935 Nov 27 1948.

The effects of treatment with Lester Smith s crystalline anti-pernicious anemia factor in 2 cases of tropical macrocytic anemia are reported. Both patients received a single injection of a solution containing 80 mcg. of the factor In each case there was a pronounced reticulocyte response, of 11.5 per cent on the sixth day in one patient, and of 36.8 per cent on the fifth day in the other Each patient also showed an increase in red blood cells and hemoglobin and change of the bone marrow from megaloblastic to normoblastic. Improvement in general condition paralleled that which occurred in the blood picture, as evidenced by greatly increased appetite and rapid gain in weight. One patient, however had a recurrence of stomatitis ten days after administration of the factor

3. GOLDSMITH, G *Vitamin B_{12}, pteroylglutamic acid and liver extract in the treatment of macrocytic anemia*, Am. J Med. 7 258, Aug. 1949 (in Soc. Proc.)

Administration of vitamin B_{12} to 6 patients and pteroylglutamic acid to 24 with macrocytic anemia in relapse was followed by an increase in reticulocytes, erythrocytes and hemoglobin. A normal or slightly subnormal blood picture was maintained in 12 patients for more than six months with 5 mg. of pteroylglutamic acid daily Doses of 2.5 mg. a day were equally effective in 2 patients with sprue and 2 with nutritional macrocytic anemia, but the blood count fell in 2 patients with pernicious anemia. Liver extract maintained the erythrocyte count and hemoglobin at higher levels than did 5 to 30 mg. of pteroylglutamic acid daily in 4 of 5 patients. Substitution of vitamin B_{12} for pteroylglutamic acid in 1 patient was followed by improvement in the blood picture.

Of 9 patients with pernicious anemia treated with pteroylglutamic acid, 2 developed a neurologic relapse during therapy while existing neurologic changes in 2 others were unaffected. Neurologic abnormalities were reversed by vitamin B_{12} in 1 patient who received this therapy

4 BETHELL, F H.: *Treatment of macrocytic anemias with vitamin B_{12}*, J Am. Dietet. A. 26 89-92, Feb. 1950.

At the Simpson Memorial Institute, 20 patients with addisonian pernicious anemia have been treated with vitamin B_{12} by intramuscular injection for periods up to 18 months. Satisfactory remissions have been maintained in each case. Recommended initial therapy for patients in relapse is 10 mcg. a day for the first week, 10 mcg. three times a week during the next two weeks, and twice a week thereafter until blood values are within normal limits. Maintenance therapy should be adjusted to the needs of the individual. If vitamin B_{12} concentrates are used instead of the pure crystalline compound, their potency should be expressed in U.S.P antianemia units.

The response of macrocytic anemias other than pernicious anemia to treatment with vitamin B_{12} has been variable. The author has observed complete failure of response to the vitamin in one patient with macrocytic anemia of the puerperium who subsequently responded to folic acid therapy (*J Lab & Clin. Med.* 33 1477 1948) but other investigators have reported good results in tropical sprue associated with macrocytic anemia and in nutritional macrocytic anemia, and in some cases of nutritional macrocytic anemia in infancy

As previously reported (Bethell *et al. Univ Hosp. Bull., Ann Arbor* 15 49 1949) vitamin B_{12} given orally alone has proved ineffective, but 5 mcg. a day in combination with extracts of hog duodenal mucosa (which have no hemopoietic activity when given alone) induce remissions. Six patients with previously untreated pernicious anemia have responded satisfactorily to this treatment.

University of Michigan
Ann Arbor, Mich.

5 GOLDSMITH, G A. *Effects of vitamin B_{12} given orally and parenterally and of a concentration of "intrinsic factor" in macrocytic anemias*, Am. J Med. 9 399 Sept. 1950 (in Soc. Proc.)

Twenty-two patients with macrocytic anemia have been treated with oral or parenteral vitamin B_{12} alone or with folic acid or a concentrate of "intrinsic factor"

to 200 mg. this substance markedly potentiated the effect of B_{12} in 9 pernicious anemia patients.

The isolation and synthesis of folic acid disclosed another substance necessary for normal hemopoiesis. Severe macrocytic anemia may develop in the absence of folic acid, but folic acid, though it causes initial remission in pernicious anemia, does not prevent neurologic degenerations or relapse in this disease. Since liver extract will arrest the neurologic disturbances, it is evident that folic acid cannot replace liver factor nor is the function of liver factor merely to make folic acid available.

How these erythropoietic substances affect cell metabolism is not well known, but there is considerable evidence that they function as enzymes in the synthesis of nucleic acid. Probably folic acid furnishes the substrate for the formation of folinic acid, or the citrovorum factor instead of being active itself. Citrovorum factor appears to facilitate formation of pyrimidines from amino acids and other simple materials, while B_{12} seems to be active in the synthesis of nucleosides from pyrimidines and purines. Theoretically citrovorum factor should be an ideal antidote for aminopterin poisoning, because it counteracts the B_{12} antagonism of aminopterin.

Vitamin B_{12} is effective parenterally in very small doses, 10 mcg. per day being a generous optimum dose during the first week of pernicious anemia therapy. Given orally B_{12} is not effective unless combined with intrinsic factor and ten times the parenteral dose is necessary. Folic acid administered alone in pernicious anemia is dangerous because it does not prevent neurologic manifestations. Folic acid may be very effective, however in macrocytic anemias caused by dietary insufficiency pregnancy sprue, and other disturbances of intestinal absorption, in which neurologic degeneration seldom occurs.

There are 19 references.

11. HORRIGAN D L., and HEINLE, R. W *Refractory macrocytic anemia with defect in vitamin B_{12} binding and with response to normal plasma*, J Lab. & Clin. Med. 40 811-812, Nov 1952 (in Soc. Proc.)

"In vivo studies in this laboratory and in vitro bone marrow culture studies by others have suggested that vitamin B_{12} is utilized for hematopoiesis in a bound form. It was postulated, therefore, that a deficiency in binding of the vitamin might result in 'refractory macrocytic anemia.

"A patient was observed who has had unexplained anemia for eighteen years which did not respond to crude liver extract given intramuscularly every week nor to iron orally Free HCl was present in fasting gastric contents. Study of hematologic remissions and relapses in this patient during the past year have provided data relative to the role of B_{12} binding in the utilization of the vitamin for hematopoiesis. Hematologic findings during relapse included macrocytic anemia, reticulocytosis, and normoblastic bone marrow hyperplasia. Remissions have been produced by a single intramuscular dose of 90 µg. of vitamin B_{12} or by the intravenous administration of 250 ml. of human plasma. Subsequent to administration of either there was a rise of hemoglobin from 9 to 12 G 100 c.c. of blood, and of the hematocrit from 31 to per cent. In either instance, remission was not mainta either with vitamin B_{12}, 30 µg. intramuscularly every to two weeks, or with oral folic acid, 10 mg. daily

"Intramuscular administration of 0.5 µg. of Co^{60} labeled vitamin B_{12} resulted in the urinary excretion of 60 per cent of the administered dose within twenty four hours. (Normal persons excreted 0 to 10 per cent under similar conditions.) After giving normal plasma, the urinary excretion of the same dose of the labeled vitamin decreased to 24 per cent. Uric acid in the erythrocytes was elevated during relapse and decreased to normal with remission. No evidence of increased blood destruction was demonstrated.

"These observations suggest that (a) the anemia in this subject results from B_{12} deficiency caused by failure of proper binding of the vitamin, and (b) normal plasma contains a factor which corrects this defect. This factor may be (a) binding substance itself, (b) a substance necessary for the combination of B_{12} and the binding substance endogenously or (c) amounts of the vitamin in bound form sufficient to promote hematopoiesis."

Cleveland, Ohio

12. BEYERS, M. R., DIEFENBACH, W C. L., MARK, H., and MEYER, L. M.: *Interrelationship of folic acid and vitamin B_{12} in macrocytic anemia associated with linitis plastica*, Acta med. scandinav 142 351 Nov 5 1952.

13. McKUSICK, V A. *Boeck's sarcoid of the stomach with comments on the etiology of regional enteritis*, Gastroenterology 23 103-113, Jan. 1953.

Two cases of Boeck's sarcoid of the stomach are described. One of the patients also had macrocytic anemia which responded dramatically to parenteral administration of vitamin B_{12}.

Johns Hopkins Hospital
Baltimore, Md.

Megaloblastic Anemia

14 UNGLEY C. C.: *Modern management of megalocytic anemias* Lancet 2 66, July 10 1948 Brit. M. J 2 154, July 17 1948.

15. DAVIDSON S *Folic acid and its conjugates in the pathogenesis of megaloblastic anaemia*, Lancet 2 814-815, Oct. 29 1949 (in Letters to the Editor)

The writer examines the data presented by Wilkinson and Israels (Oct. 15) from which they concluded that patients with addisonian pernicious anemia have a normal capacity to release folic acid from its conjugates and that folic acid or its conjugates play no major part in the etiology of the human pernicious anemia syndrome. He finds these deductions unwarranted, and believes that present knowledge indicates a requirement of both folic acid and vitamin B_{12} for prevention of megaloblastic blood formation.

University of Edinburgh
Edinburgh, Scotland

16. MUELLER, J F., HAWKINS, V R., and VILTER, R. W *Liver extract refractory megaloblastic anemia*, Blood 4 1117 1123, Oct. 1949

This is a case study of a patient with macrocytic anemia, megaloblastic maturation arrest in the bone marrow glossitis, hyper reflexia and diminished vibration perception in the feet. Vitamin B_{12} given parenterally (8 mcg. initially and on each of three successive days) and liver extract produced no improvement in any respect. Folic acid, however, in a dosage of 7.5 mg. a day intra muscularly for 10 days, had a favorable effect on all the abnormalities except the neurologic signs. The patient appeared to have the type of megaloblastic anemia that is described as "achrestic anemia" and "refractory megaloblastic anemia." It resembles "Wills factor deficiency anemia" and some cases of pernicious anemia of pregnancy Folic acid deficiency apparently was not the primary etiologic factor since the urinary excretion of folic acid was within normal limits. Deficiency of an unknown factor probably the equivalent of the "Wills factor" is suggested. It seems likely that folic acid overcame deficiency of this factor by a "mass action," which may also explain its hemopoietic action in pernicious anemia. A later hematologic response to thymine which occurred when the case described relapsed, is consistent with such a theory

17 SCHIATOLLA, E., GIBSON, A., and CARLISLE, J M.: *Crystalline vitamin B_{12} in the treatment of megaloblastic anemias*, Postgrad. Med. 6 303-310 Oct. 1949 Mod. Med., Oct. 1 1949 pp. 60-64.

This article is adapted from the Scientific Exhibit presented by members of the Medical Division of Merck & Co., Inc., at the 1949 convention of the American Medical Association at Atlantic City Crystalline vitamin B_{12}, which was first isolated in the Merck laboratories early in 1948, is depicted in color This is a pure anti-anemia substance of high potency in the treatment of anemias characterized by erythrocyte maturation arrest. Vitamin B_{12} has also been shown to be an essential "growth factor" for certain species of animals.

The pathogenesis of the megaloblastic anemias and the diagnostic criteria for pernicious anemia are shown pictorially The bone marrow and blood responses to vitamin B_{12} therapy are illustrated in color while a chart shows the improvement in neurologic complications obtained by this treatment.

18. MILLER, C. M., and MOORHOUSE, E. H. *Treatment of megaloblastic anaemia with vitamin B_{12}* Brit. M. J 2 1511 1512, Dec. 31, 1949

A satisfactory clinical and hematologic response was obtained in each of 2 cases of megaloblastic anemia with vitamin B_{12} prepared from Streptomyces (Glaxo Laboratories) The patients received 20 mcg. initially by intramuscular injection and the same amount at each of a series of injections several days later the total dosages being 100 and 140 mcg. Continued treatment was impossible because of the limited supply of B_{12}.

Smithdown Road Hospital Liverpool, England

19. UNGLEY C. C. *Vitamin B_{12} in megaloblastic anaemias*, Lancet 1 930, May 13, 1950 (in Letters to the Editor)

The letter reads: "In referring to the effect of vitamin B_{12} in megaloblastic anaemias without gastric atrophy Dr Tuck and Dr Wittaker (April 22, p. 757) incorrectly abbreviate a report of my remarks at the British Association meeting last year which was already too condensed to be accurate. These anaemias are a 'mixed bag and the response to vitamin B_{12} varies widely It is true that megaloblastic anaemias of pregnancy and the puerperium in temperate climates did not respond, but Patel and Kocher report good results in a tropical variety of the syndrome.

"Megaloblastic anaemias associated with intestinal disorders—even those encountered in this country—have not always failed to respond to vitamin B_{12}. On the other hand, a state refractory to vitamin B_{12} may exist temporarily even in a patient presumed to have gastric atrophy Such cases are rare. In one instance the anaemia became responsive to vitamin B_{12} only after small amounts of folic acid had been supplied. This recalls the pigs of Heinle, Welch, and Pritchard, which were so depleted of haemopoietic factors that folic acid had to be given before liver extract would work, and vice versa."

Royal Victoria Infirmary Newcastle upon-Tyne, England

20. UNGLEY C. C. *Vitamin B_{12} and other dietary factors in megaloblastic anaemias*, Brit. J Nutrition 4 252 259 1950.

21. ROYAL SOCIETY OF MEDICINE: *Pathogenesis and treatment of megaloblastic anaemias*, Brit. M. J 1 1367 June 10, 1950 (in Soc. Proc.)

22. FOY H., KONDI, A., and HARGREAVES, A.: *The megaloblastic anaemias*, Lancet 1 1172 1173, June 24 1950 (in Letters to the Editor)

This communication is a discussion of the nature and etiology of the megaloblastic anemias. The writers believe that the megaloblastic macrocytic anemias have a different etiology from the megaloblastic normocytic anemias and believe the presence or absence of the giant stab cells to be significant. They cite the responses of the several types of anemia to folic acid and vitamin B_{12} and try to relate these differences to their discussion.

Nairobi, Kenya Africa

23. PATEL, J C. *Vitamin B_{12}*, Lancet 2 407 Sept. 23, 1950 (in Soc. Proc.)

"Dr J C. Patel (India) had found among patients with tropical megaloblastic anaemia that those with free HCl in the gastric juice often respond to B_{12} orally in doses of 20-600 µg. daily some of the achlorhydrics showed an irregular response, but in true pernicious anaemia there was no response at all."

24 UNGLEY C. C. *Vitamin B_{12}. Part 2. A review of the clinical aspects* Nutrition Abstr & Rev 21:1-26, July 1951 (abstr J Am. Dietet. A. 28 160, Feb. 1952)

"This review of the clinical aspects of vitamin B_{12} is concerned primarily with the effects of oral and par enteral administration of the vitamin to human subjects suffering from megaloblastic anemias. Current views of the role of Castle s intrinsic factor in the absorption and utilization of the vitamin are presented also, along with a discussion of its fate after oral and parenteral administration."

25 GIRDWOOD R. H.: *Vitamin B_{12} and folic acid in the megaloblastic anaemias* Edinburgh M J 58 309-335, July 1951.

26. WITTS, L. J *Pathogenesis of the megaloblastic anaemias*, Lancet 2 367-369 Sept. 1 1951.

27 UNGLEY C. C.: *Megaloblastic anaemias*, Lancet 2 1068, Dec. 8, 1951 (in Soc. Proc.)

In discussion at a meeting of the Royal Society of Medicine, "Dr C. C. Ungley suggested that the gastric at rophy that was the classical feature of pernicious anaemia led not only to a nutritional defect and loss of vitamin B_{12}, but also, by changes in the bacterial flora of the upper intestine, to the formation of 'toxic factors. With the help of diagrams he showed how these two factors could produce the known syndromes of megaloblastic anaemia, but he gave warning that these diagrams were as yet theoretical and that the experimental basis for many of the reactions shown was still missing or unconfirmed. Dr Ungley believed that both folic acid and vitamin B_{12} were concerned in normal erythropoiesis folic acid accelerated the utilisation of vitamin B_{12}, so that in pernicious anaemia the action of folic acid fell off when remaining stores of vitamin B_{12} were exhausted. He quoted experiments show ing that small doses of vitamin B_{12} would act when given orally only if it was previously treated with normal gastric juice. It had been suggested that gastric juice acted by preventing the bacteria in the intestine from metabolising vitamin B_{12}, so that when there was achylia B_{12} in the food was taken up by the bacteria and did not reach the erythropoietic tissues. Dr Ungley 'sterilised' the intestinal tract of a patient by giving Aureomycin, and he found that 80 μg. of vitamin B_{12} given orally was still ineffective, though after incubation with normal gastric juice 80 μg. produced a good response as before. He believed, therefore, that the 'intrinsic factor in gastric juice actually combined with vitamin B_{12} to form a new factor that could pass the intestinal mucosa, but there was evidence that the combination was split up again and the vitamin B_{12} freed in the body fluids after absorption. There seemed to be a logarithmic relation between the parenteral dose of vitamin B_{12} and the rise of the red-cell count in the first 15 days after administration of up to 320 μg.; bigger doses gave less than the expected response."

28. GIRDWOOD R. H.: *The interrelationships of factors that influence the megaloblastic anemias* Blood 7 77-93 Jan. 1952.

Author s conclusions after reviewing 112 references "This review of a vast literature is necessarily incomplete, and it is possible that investigations that may eventually prove to be of great importance have been omitted. The work reviewed here indicates that vitamin B_{12}, pteroylglutamic acid and the citrovorum factor are probably concerned with the metabolism of the maturing red quite apart from their other undoubted metabolic which have not been considered. Pteroylglutamic and vitamin B_{12} play a part in methyl metabolism and the metabolism of glycine, serine, ethanolamine and formic acid, all of which are probably concerned both with the formation of purines and pyrimidines and also with the synthesis of porphyrins. This, too may be significant in relation to blood metabolism.

"Bone marrow cultures have suggested that substances may exist in the serum that inhibit normal blood formation. If this is true of what takes place in the body we do not know the mode of formation of such inhibitors, or whether any part of the action of the antimegaloblastic factors is concerned with the overcoming of such inhibitors.

"Two main theories about the interrelationships of these factors have been considered. The view that vitamin B_{12} is concerned with the release of pteroylglutamic acid from its conjugates is unsatisfactory mainly because it does not explain why pernicious anemia patients cannot be maintained indefinitely in hematologic remission with synthetic pteroylglutamic acid. It must be admitted, however that there still exists the possibility that one of the actions of vitamin B_{12} is to do this.

"The other theory put forward in part by Wright, Skeggs and Huff and elaborated by Vilter et al. is based very largely upon bacteriologic findings and requires further investigation in relation to megaloblastic anemias in man. According to this theory pteroylglutamic acid which may be temporarily effective in a dosage of as little as 1 mg. daily acts by a 'mass action effect in pernicious anemia. Several workers have suggested that if it does act in such a way traces of vitamin B_{12} in the body may be used up in the process. There is some evidence that vitamin B_{12} may not entirely be lacking from the tissues in pernicious anemia. Subacute combined degeneration of the cord, the pathogenesis of which is uncertain, might then arise when vitamin B_{12} depletion was complete or almost complete.

"Finally there is evidence for the presence of numerous forms of vitamin B_{12} in the body and to the complexities of the interrelationships of the various factors there is added the complexities of the metabolic interrelationships of the various forms of vitamin B_{12}."

University of Edinburgh
Edinburgh, Scotland

29 HARRISON H. E., in discussion on May C. D., Hamilton, A., and Stewart, C. T: *Folic acid deficiency experimental and clinical*, A.M.A. Am. J Dis. Child. 84 475-477 Oct. 1952 (in Soc. Proc.)

"I have observed a baby with megaloblastosis who apparently had a congenital deficiency of absorption of vitamin B_{12}. This child had a characteristic picture of megaloblastic anemia but responded not at all to folic acid, even in extremely large doses or in doses given intramuscularly but did respond in typical fashion to vitamin B_{12} given intramuscularly and to very large doses given orally The child had a relapse every time administration of vitamin B_{12} was stopped."

30. UNGLEY C. C. *The pathogenesis of megaloblastic anaemias and the value of vitamin B_{12}*, Brit. J Nutrition 6 299-315 (No. 3) 1952.

31. THEDERING F., and RIETHMUELLER: *Vitamin B_{12} and folic acid in the treatment of megaloblastic anemia*, Folia clin. internacional 2 371 1952 (abstr Semana méd. 102 96, Jan. 15 1953)

The authors recommend combined therapy of vitamin B_{12} and folic acid for pernicious type anemia. If it is classic type pernicious anemia, vitamin B_{12} alone or in combined therapy is effective, but in some megaloblastic anemias, combined therapy has been found more effective. The original article is described as containing a review of the literature and the authors clinical experience.

32. GIRDWOOD R. H. *The relationships between vitamin B_{12}, folic acid and folinic acid*, Brit. J Nutrition 6 315-324 (No. 3) 1952 (abstr J Am. Dietet. A. 29 160, Feb. 1953)

"Experiments indicate the importance of supplying both vitamin B_{12} and folic acid to prevent megaloblastic anemia in the pig. Protein deficiency increases the need for folic acid. Monkeys placed on a low-protein diet have been found to develop megaloblastic anemia more rapidly if there were also an ascorbic acid deficiency When anemia did develop folic acid was rapidly effective in treatment. Ascorbic acid was more slowly effective and vitamin B_{12} was ineffective, but B_{12} plus ascorbic acid gave a prompt response. The normal daily intake of folic or folinic acid required to prevent nutritional megaloblastic anemia in man is uncertain. All that can be concluded at present is that vitamin B_{12} and folic acid both appear to be required for normoblastic blood formation, and that ascorbic acid deficiency appears to be related to the development of megaloblastic anemias."

PERNICIOUS ANEMIA

33. WEST R.: *Activity of vitamin B_{12} in addisonian pernicious anemia*, Science 107 398, April 16, 1948.

Vitamin B_{12}, the new crystalline compound isolated from liver has produced positive hematologic responses in 3 patients with pernicious anemia following single intramuscular injections of 3, 6, and 150 mcg., respectively Four similar patients given single injections of amorphous liver concentrates containing 20,000 to 40,000 LLD units of the compound showed strong or maximal hematologic responses, while 3 patients given concentrates containing only 10,000 LLD units or less manifested little or no response. In the 3 patients given crystalline vitamin B_{12} there has been a rise in reticulocytes, red cell count, and hemoglobin, but it is still too early to say whether the blood picture will return to normal without further treatment. In one of these patients there has been a striking rise in the white blood cell count, the platelets have risen from 120,000 to 340,000. It is of interest that Preparation No. 31 of Dakin, Ungley and West (*J Biol. Chem.* 115 771, 1936) which was clinically active, has been shown by microbiologic assay to contain about 30,- LLD units (3 mcg.) of vitamin B_{12} per 25 mg., which is the dose used at the time.

Columbia University College of Physicians and Surgeons, and Presbyterian Hospital New York, N. Y.

34. STOKSTAD E. L. R., PAGE, A., JR., PIERCE, J., FRANKLIN A. L., JUKES, T. H., HEINLE, R. W., EPSTEIN, M., and WELCH, A. D *Activity of microbial animal protein factor concentrates in pernicious anemia*, J Lab. & Clin. Med. 33 860-864, July 1948.

A nonmotile, rod-shaped organism from hen feces has been found to produce an animal protein factor which is necessary for chick growth and which is capable of producing hematologic and clinical responses in pernicious anemia. Two concentrates of the growth media, one prepared by clarification and precipitation with ammonium sulfate (concentrate I) and the other by clarification alone (concentrate II) have been tested clinically Concentrate I on chick assay had between 50 and 100 per cent the activity of a liver extract containing 10 antipernicious anemia units per cc., and on Streptococcus fecalis assay contained only 0.5 mcg. of folic acid per cc. Concentrate II had between 25 and 40 per cent the activity of a 10-unit liver extract and contained about 0.02 mcg. of folic acid per cc. Treatment with "conjugase" did not increase the folic acid value of either concentrate.

Concentrate I was given in daily intramuscular doses of 1 cc. for nine days to a patient 90 years of age suffering from pernicious anemia, with severe nausea and fecal vomiting. After the third injection nausea and vomiting ceased and did not reappear The reticulocyte response was less prompt than the usual response to effective intramuscular liver therapy but the level reached was nearly maximal the rise began on the fourth day and reached a peak of 20.6 per cent on the tenth. This peak was followed by a prompt increase in erythrocytes, leukocytes, and platelets, and in hemoglobin, with corresponding clinical improvement. After an interval of nine days a course of liver therapy was given, 1 cc. of a 10-unit extract being administered intramuscularly each day for nine consecutive days. A second, small reticulocyte peak, 6 per cent, occurred on the sixth day The patient continued to improve.

Concentrate II was given to another aged patient with pernicious anemia. The dosage was the same as with concentrate I in the previous case, but treatment was continued for thirty days. The reticulocyte response was submaximal, a peak of 9 7 per cent occurring on the seventh day There was a prompt increase in erythrocytes and leukocytes and in hemoglobin. After thirty days, the erythrocyte count had risen by more than 1,500,000 cells per cu. mm. and the hemoglobin from 7 7 to 10.8 Gm. per 100 cc. The mean corpuscular volume and mean corpuscular hemoglobin showed a decrease.

Whether this new factor is identical with the antipernicious anemia factor in liver extracts or with the recently isolated vitamin B_{12} which is active in pernicious anemia has not yet been determined.

35. ADDINALL, C. R. *Recent advances in the chemistry of natural products the isolation and properties of vitamin B_{12}*, Merck Report 57 4-7 Oct. 1948.

The author traces the course of therapy for pernicious anemia from liver substance, through liver extract, folic acid, and thymine to its present culmination in vitamin B_{12}.

Merck & Co., Inc. Rahway N J

36. BETHELL, F H., in discussion on Haden, R. L., and Bortz, D W *Treatment of idiopathic pernicious anemia*, J.A.M.A. 138 870-873 Nov 20 1948.

Dr Bethell discusses an article in which complete liver extract is described as the treatment of choice in pernicious anemia. He states that vitamin B_{12} may lead to complete revision of the specific treatment of pernicious anemia and related anemias. He has used vitamin B_{12} and it has induced optimal hemopoietic responses in patients with pernicious anemia, even when given in doses as small as 1 mg. [1 microgram?] daily The number of patients receiving the vitamin is not stated.

37 HALL, B. E., and CAMPBELL, D C.: *Vitamin B_{12} therapy in pernicious anemia. I Effect on hematopoietic system preliminary report*, Proc. Staff Meet., Mayo Clin. 23: 584-590, Dec. 8, 1948.

The effect on the hemopoietic system of vitamin B_{12} given intramuscularly was studied in 11 patients with pernicious anemia in relapse. The reticulocyte response and the rate of rise in erythrocyte levels were comparable to those obtained when liver is employed. The reticulocyte peak occurred on the fourth to the seventh day after institution of therapy In some cases the erythrocyte count rose to normal in five to seven weeks and in others it leveled off after it had exceeded 3,000,000/cu. mm. of blood and did not rise further for one to three weeks.

The effect of approximately 1 microgram of vitamin B_{12} was equivalent to that of 1 U.S.P unit of extracts of liver or stomach mucosa. Various dosages were employed. In some cases 1 mcg. a day induced maximal hemopoietic responses. Weekly doses of 25 mcg gave excellent hemopoietic responses in 3 patients, but in 2 of them the erythrocyte count ceased to rise when the interval between injections was prolonged beyond 12 days. A satisfactory response was noted in 1 case from intramuscular administration of 5 mcg. of vitamin B_{12} for five days and then of 5 mcg. three times a week. Maintenance doses have not yet been established.

Serial aspirations of the sternal marrow have shown regeneration of erythrocytes from megaloblastic to normoblastic types of cells in 48 to 72 hours after administration of relatively large doses of vitamin B_{12}. In one case normoblastic regeneration was virtually complete 48 hours after the administration of a single injection of 25 mcg.

38. HALL, B. E., and CAMPBELL, D C. *Effect of vitamin B_{12} on the hematopoietic and nervous systems in addisonian pernicious anemia*, J Lab. & Clin. Med. 33 1646, Dec. 1948 (in Soc. Proc.)

Six patients with pernicious anemia in relapse, 4 of whom had not received prior treatment, were treated with vitamin B_{12}. Doses of 25 mcg. given intramuscularly at weekly intervals produced excellent hematologic responses. When the interval between injections was more than a week the rise in erythrocytes was not always so great. Reticulocyte peaks were noted four to seven days after initiation of vitamin B_{12} therapy and normal erythrocyte counts were reached in four to six weeks. Complete conversion of the bone marrow from megaloblastic to normoblastic regeneration occurred 48 to 90 hours after administration of vitamin B_{12}.

Glossitis was relieved by vitamin B_{12} in the 4 cases in which it was present. Four of the 6 patients had subacute combined degeneration of the spinal cord. There was remarkable improvement in one of these patients who received 75 mcg. of vitamin B_{12} over a period of 33 days but another showed no subjective or objective improvement after receiving 100 mcg. over a period of 62 days the remaining 2 are still under observation. The necessity for observation over many months to determine whether vitamin B_{12} will permanently protect the central and peripheral nervous systems in pernicious anemia is stressed

39 CLOUGH, P W: *Further refinement of liver extracts effective in pernicious anemia*, Ann. Int. Med. 29: 1169-1172, Dec. 1948 (Editorial)

40 QUERIES AND MINOR NOTES: *Vitamin B_{12} for pernicious anemia*, J.A.M.A. 142 297 Jan. 28, 1950.

The editor is asked whether vitamin B_{12} therapy entirely eliminates the need for liver therapy in patients with pernicious anemia. In reply it is stated that according to present indications, vitamin B_{12} is effective in controlling both the blood and neurologic changes in patients with pernicious anemia. It is not known whether liver extract has any additional properties or not. It would appear to be safe to use vitamin B_{12} injections without liver extract if proper blood and neurologic studies are made at intervals to determine whether there are any deleterious changes. A dosage of 10 mcg. of vitamin B_{12} per week is recommended.

41 SPIES, T D., STONE, R. E., KOCH, M. B., GRANT H. M., and MOORE, M. M. *The hemopoietic response of patients with pernicious anemia to crystalline vitamin B_{12}*, South. M. J 43 50-51, Jan. 1950.

Four patients with acute pernicious anemia in relapse showed a satisfactory hemopoietic and clinical response to vitamin B_{12}. One case history is reported this patient responded to a single intramuscular dose of 10 mcg. of B_{12}. The reticulocytes reached a peak of 16 per cent on the tenth day after therapy and subsequently there was an increase in red blood cells and hemoglobin. Clinical response preceded the hemopoietic response.

Northwestern University Medical School
Chicago, Ill.
Hillman Hospital
Birmingham, Ala.

42. BERGER, M. *Vitamin B_{12} in addisonian pernicious anemia in a liver sensitive person*, New York State J Med. 50 331-332, Feb. 1, 1950.

A woman with addisonian pernicious anemia developed sensitivity to liver Folic acid controlled the hematologic situation, but symptoms of combined system disease developed. Treatment was changed to 10 mcg. of vitamin B_{12} once a week, and improvement in the neurologic symptoms was evident within five days. After six weeks of treatment there was no subjective or objective evidence of combined system disease. At this point the supply of vitamin B_{12} was exhausted, and it was necessary to substitute folic acid treatment. Neurologic symptoms returned under this treatment. Vitamin B_{12} is particularly valuable in that it apparently contains factors effective against both anemia and combined system sclerosis.

An addendum states that since these observations were made another investigator has reported that vitamin B_{12} is not efficacious in the treatment of the megaloblastic anemias of infancy unless minute amounts of folic acid are present. This suggests that folic acid plays the role of a catalyst in this disease.

Richmond Memorial Hospital and Berger Clinic
Tottenville, N Y

43 CHALMERS, J N M.: *Recent work on vitamin B_{12}*, Lancet 1 354 Feb. 25, 1950 (in Soc. Proc.)

In a discussion of vitamin B_{12} in the anemias Dr Chalmers reported that "in his clinic a maintenance dose of 20-40 µg. fortnightly had mostly proved satisfactory in a few cases 60 µg. had been needed. In his experience vitamin B_{12} did everything that liver did and it had proved useful in patients who had relapsed on unsatisfactory liver extract. Eight cases treated with the 'unnamed substance' were doing well. Vitamin B_{12} was satisfactory in 4 patients allergic to liver extract 1 of these who suffered from crippling subacute combined degeneration, was restored to actively earning a living."

44 KINNEAR, T., and HUNTER, R. B. *Therapeutic potency of vitamin B_{12} derived from Streptomyces griseus culture liquors*, Edinburgh M. J 57 65-71, Feb. 1950 (abstr J.A.M.A. 143 1288-1289 Aug. 5, 1950)

The authors treated 34 pernicious anemia patients with vitamin B_{12} obtained from Streptomyces griseus liquors. The authors believe that vitamin B_{12} derived from Streptomyces liquors is satisfactory for treating pernicious anemia and for maintenance therapy There is as yet no proof that vitamin B_{12} will prevent the onset of subacute combined degeneration, but the evidence so far suggests that this condition does not progress when therapy is adequate, and in no case has it developed during treatment.

45. LEADING ARTICLE *Vitamin B_{12} and pernicious anaemia*, Lancet 1 500-501, March 18, 1950.

It is stated that vitamin B_{12} is effective in producing and maintaining remission in pernicious anemia. Several hypotheses concerning the etiology of pernicious anemia are discussed, and evidence is cited to support or disprove them. It is concluded that any practical hypothesis concerning the etiology of pernicious anemia must take into account the invariable achlorhydria and failure of secretion of the gastric enzymes, including intrinsic factor Vitamin B_{12} has properties resembling known extrinsic factors; however it is probably not the only source thereof.

46. HARTLEY F *Vitamin B_{12} and pernicious anemia*, Lancet 1 594, March 25, 1950 (in Letters to the Editor)

The writer believes that the following statement (from a leading article in the March 18 *Lancet* [Abstr 45]) needs qualification "There is now sufficient evidence for us to say that preparations of vitamin B_{12} from liver or Streptomyces griseus will produce a remission in pernicious anemia, and that regular dosage will maintain a normal blood picture." He states that in many cases vitamin B_{12} is ineffective.

The British Drug Houses, Ltd.
London, England

47 UNGLEY C. C.: *Vitamin B_{12} and pernicious anemia*, Lancet 1 593-594, March 25, 1950 (in Letters to the Editor)

The writer feels that certain references to his work in a leading article in the March 18 *Lancet* (Abstr 45) require qualification. He states that 10 mcg doses of vitamin B_{12} given every two weeks, are adequate in some, but not in all, cases of pernicious anemia in producing satisfactory increases in red cells or hemoglobin. Those patients whose blood is examined only once in six months are usually given 30 to 60 mcg. every three weeks. Larger doses are given to patients with subacute combined degeneration. The writer further states that a mixture of 5 mcg. of vitamin B_{12} with 50 cc. of human gastric juice given daily has approximately the same hemopoietic effect as a single dose of 10 mcg. of vitamin B_{12} given parenterally This relationship varies appreciably from case to case.

Royal Victoria Infirmary
Newcastle-upon-Tyne England

48. GIRDWOOD R. H.: *Vitamin B_{12} and pernicious anaemia*, Lancet 1 594, March 25, 1950 (in Letters to the Editor)

In none of 3 patients with pernicious anemia in relapse nor in 2 controls were there any appreciable amounts of vitamin B_{12} or folic acid in the small intestine. In these 5 subjects a day's stools yielded about 5 mcg. of vitamin B_{12} and 0.5 mg. of folic acid. In a patient with ulcerative colitis, each cubic centimeter of material obtained from the lower end of the ileum contained about 0.03 mcg. of growth factors for L. leichmannii and 12 mcg. of folic acid. It was considered improbable that relapses and remissions in pernicious anemia could be related to changes in the flora of the small intestine.

University of Edinburgh
Edinburgh, Scotland

49 WATSON, J., LICHTMAN H., GINSBERG V., PIERCE, J V., STOKSTAD E. L. R., and JUKES, T H.: *Use of vitamin B_{12b} in pernicious anemia*, Am. J Med. 8 398, March 1950 (in Soc. Proc.)

A solution of crystalline vitamin B_{12b}, 10 mcg. per cc., was prepared in 0.9 per cent sodium chloride and sterilized by filtration. This material was administered to 4 patients with addisonian pernicious anemia. Of 2 who were given 1 mcg. daily intramuscularly one responded with a reticulocyte peak and a red cell rise higher than expected, and the other gave a submaximal reticulocyte response with a maximal red cell rise at three weeks. Hematologic response was satisfactory in the remaining 2 patients, who were given 1.5 and 2 mcg. daily by injection, respectively Clinical improvement, including neurologic, was excellent in all 4 patients. It is concluded that 1 to 2 mcg. of vitamin B_{12b} daily by intramuscular injection is hemopoietically equivalent to one U.S.P unit of liver extract and is comparable in potency to vitamin B_{12}.

Another patient who had pernicious anemia of pregnancy failed to respond to large amounts of liver extract, then failed to respond to 1 mcg. of vitamin B_{12}, daily but showed a dramatic response to the subsequent daily injection of 15 mg. of folic acid.

The oral administration of vitamin B_{12}, is being studied.

Kings County Hospital
Brooklyn, N Y
Lederle Laboratories
Pearl River N Y

50. MOLLIN D L.: *Vitamin B_{12} and pernicious anaemia*, Lancet 1 690, April 8, 1950 (in Letters to the Editor)

The writer reports the treatment with vitamin B_{12} of 19 successive patients suffering from pernicious anemia. Remissions obtained were similar to those with the best liver extracts.

London, England

51. ERF L. A., and WIMER, B. M. *Pernicious anemia discussion of treatment with special reference to vitamin B_{12}*, GP 1 59-66, April 1950.

52. MEYER, L. M., SAWITSKY A., COHEN B. S., KRIM, M., FADEM, R., and RITZ, N D: *Oral treatment of pernicious anemia with vitamin B_{12}*, Bull. New York Acad. Med. 26 263-264, April 1950 (in Soc. Proc.)

Five patients with pernicious anemia in relapse were treated with daily oral doses of 75 to 150 mcg. of vitamin B_{12} for periods up to seven months. Reticulocytosis was submaximal but clinical and hematologic response was satisfactory and a patient with evidence of combined system disease showed improvement in neurologic status. Parenteral administration of vitamin B_{12} was effective in 2 patients who had not responded to daily oral dosage of 150 and 250 mcg., respectively Two patients had a suboptimal reticulocyte response, but satisfactory hematologic response and clinical improvement followed administration of 2 Gm. of desiccated hog duodenum mucosa and 10 mcg. of vitamin B_{12} daily for two months. Two additional patients were given daily oral doses of 1.67 mg. of folic acid and 25 mcg. of vitamin B_{12} for five weeks. Reticulocytosis was maximal and the rise in hemoglobin and erythrocytes was rapid. Increase of leukocytes above 5,000 per cc. mm. was slow Clinical improvement was early and progressed favorably In 1 patient glossitis disappeared in a week and neurologic symptoms soon regressed. A third patient who has recently been placed on the same treatment shows a similar clinical and hematologic response and an optimal reticulocytosis.

Bronx Veterans Hospital, and
Goldwater Memorial Hospital
New York, N Y

53. MEYER, L. M., KRIM, M., and SAWITSKY A. *Oral treatment of pernicious anemia with swine duodenal mucosa and vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 73 565-568, April 1950.

Two cases are reported confirming Bethell's finding that the ingestion of desiccated swine duodenal mucosa and vitamin B_{12} is effective in the oral treatment of pernicious anemia. The daily dosage in these cases was 2 Gm of an aqueous extract of swine duodenal mucosa (given for a week before vitamin B_{12} was begun, to determine whether this extract had any effect when administered alone) and 10 mcg. of vitamin B_{12}. In both cases, a suboptimal reticulocyte response was obtained and hematologic and clinical improvement both were satisfactory

Goldwater Memorial Hospital
Welfare Island
New York, N Y

54 MEYER, L. M., SAWITSKY A., RITZ, N D., and KRIM, M.: *Oral treatment of pernicious anemia with subminimal doses of folic acid and vitamin B_{12}*, Am. J Clin. Path. 20 454-457 May 1950.

Although the minimal effective oral dose of vitamin B_{12} has been found to be 75 to 150 mcg. a day the combination of 25 mcg. of vitamin B_{12} a day and 1.67 mg. of folic acid produced good hematologic response and clinical improvement in 3 patients with pernicious anemia in relapse. Folic acid acted like "intrinsic factor" in that it promoted utilization of subminimal oral doses of B_{12}.

An addendum reports 3 additional patients who have responded well to this treatment. In one case the dosage was the same as in the first 3 patients; in the other 2, 0.67 mg. of folic acid and 10 mcg. of vitamin B_{12} daily by mouth produced optimal reticulocytosis and rapid rise in hemoglobin and erythrocytes. Signs of clinical remission occurred within three days after treatment was begun.

New York University College of Medicine
New York, N Y

55. MEACHAM, G C., VIGNOS, P J., HEINLE, R. W., WEISBERGER, A. S., and EPSTEIN M. *Vitamin B_{12} concentrate in the maintenance of pernicious anemia*, J Lab. & Clin. Med. 35 713-720 May 1950.

Thirty patients with addisonian pernicious anemia were treated with vitamin B_{12} concentrate for periods of 5 to 14 months. In the dose used (1 mcg. daily by intramuscular injection every three to four weeks) the vitamin B_{12} concentrate did not maintain optimal blood levels. In 20 of the 30 patients erythrocyte counts under 4,000,000 were observed at the end of the treatment period. Of 26 patients previously treated with folic acid or liver extracts, 17 had erythrocyte decreases of over 500,000 per cc. during treatment with vitamin B_{12} concentrate.

The authors consider several explanations for the failure to maintain optimal blood levels. The most probable one is that an average dose of 1 mcg. daily is not sufficient.

In no patient did neurologic or lingual relapses develop even though blood levels were not optimal. One patient who had pronounced neurologic symptoms at the start of therapy was considerably improved after treatment with the B_{12} concentrate. No toxic or allergic symptoms due to the B_{12} concentrate were observed.

Western Reserve University School of Medicine, and
University Hospitals
Cleveland, Ohio

56. LAMBLING A., CONTE, M., DEBRAY J., and PÉQUIGNOT G. *Deux maladies de Biermer traitées par la vitamine B_{12} (Two cases of Biermer's disease treated with vitamin B_{12})* Bull. Soc. Méd. hôp. Paris 66 722-727 May 1950.

57 BERTRAND-FONTAINE, T., MALLARME, J., and SCHNEIDER, J. *Six cas de maladie de Biermer traitées par la vitamine B_{12} (Six cases of Biermer's disease treated with vitamin B_{12})* Bull. Soc. Méd. hôp. Paris 66 674-684, May 1950.

58. MORISSETTE, L.: *A propos de B_{12} (Regarding B_{12})* Union méd. du Canada 79 534-536, May 1950.

The author reports satisfactory hematologic results in a case of pernicious anemia treated with vitamin B_{12}. In a neuroanemic syndrome with little anemia, the response was very encouraging.

The author thinks that B_{12} should be reserved for the treatment of macrocytic anemias because it is less effective than liver as a general tonic and is therefore of less value in the treatment of hypochromic and other secondary anemias.

Notre-Dame Hospital, Montreal, Canada

59 SEIFE, M., and REICH, C. *Effect of vitamin B_{12} administered subcutaneously* New York State J Med. 50 1401 1402, June 1, 1950.

Almost complete remission of pernicious anemia in relapse was obtained in 2 patients who were given vitamin B_{12} subcutaneously; the usual dosage was 25 mcg. once a week. Total dosages of 95 and 130 mcg. of vitamin B_{12} were given in these two cases.

Response was as rapid as that obtained from intramuscular administration of vitamin B_{12} or liver and there was no pain or reaction from the injection. An addendum reports 2 other cases successfully treated with this method.

City Hospital, New York, N. Y.

60. UNGLEY C. C.: *Thymidine and vitamin B_{12} in pernicious anemia,* Lancet 1: 164-165, Jan. 22, 1949

A patient with addisonian pernicious anemia did not respond to thymidine but responded to a small dose of the red crystalline anti-pernicious anemia factor

61. HALL, B. E., MORGAN E. H., and CAMPBELL, D C.: *Oral administration of vitamin B_{12} in pernicious anemia. I Presence of intrinsic factor in Berkefeld filtered pooled human gastric juice preliminary report,* Proc. Staff Meet., Mayo Clin. 24 99-107 Feb. 16, 1949

Experiments with the oral administration of vitamin B_{12} in 4 cases of pernicious anemia in relapse are reported. A single oral dose of 25 mcg. in one case and 5 mcg. daily in another failed to produce a hemopoietic response. Similarly Berkefeld filtered pooled human gastric juice, from patients with uncomplicated duodenal ulcer or functional complaints referable to the gastrointestinal tract, administered orally in a quantity of 150 cc. a day for six days, appeared to be devoid of hemopoietic activity However a hemopoietic response was obtained in each of 3 patients who were given the filtered gastric juice simultaneously with, or within two hours of, the oral administration of 5 mcg. of the vitamin. Gastric juice obtained from the sources described above and passed through a Berkefeld filter must, therefore, contain the intrinsic factor The hemopoietic response was obtained when 150 cc. or 25 cc. of gastric juice was administered, but not when the quantity was reduced to 5 cc.

Although the potentiation of orally administered vitamin B_{12} by an intrinsic factor in gastric juice appears to be established, the authors believe that until the vitamin has been administered orally in much larger amounts than have so far been possible, the conclusion that this vitamin has no hemopoietic activity in pernicious anemia when administered alone is not justified.

62. JONES, E., TILLMAN C. C., and DARBY W J.: *Observations on relapses in pernicious anemia,* Ann. Int. Med. 30 874-880, Feb. 1949

Of 12 patients with pernicious anemia that had responded to liver extract therapy alone, 6 had no hematologic relapse over a period of 26 to 29 months after the treatment was discontinued. The other 6 suffered relapses only after 8 to 18 months without further treatment. Standards for hematologic relapse are described which relate relapse to the individual patient's blood level before treatment was stopped. Output of urobilinogen in the stools increases early in hematologic relapse and appears to increase further as the anemia progresses. This indicates that hemolysis becomes operative early in the relapse of pernicious anemia and emphasizes its importance in the pathogenesis of the disease.

Nashville, Tenn.

63. WALDENSTROM, J: *Vitamin B_{12},* Nord. med. 20: 887-890, March 30, 1949

Author's summary: "A brief account of the development of our theoretical knowledge about liver extracts, folic acid and B_{12} is given. The clinical results obtained with the newly isolated cobalt-containing anti-pernicious anemia factor from liver (Vitamin B_{12}; trade name of the product from Merck: Cobione) are summarised. The excellent therapeutic effect in pernicious anemia even of extremely small doses is evident. It seems probable that this substance is of importance also for the metabolism of the nervous system as it has been observed that it heals recent nervous lesions in pernicious anemia. Its protective and general therapeutic value remains to be established. Nine cases of pernicious anemia in Uppsala, who had become sensitized against liver extracts and showed positive cutaneous tests with several such extracts, showed no reaction with B_{12}. It seems probable that the future therapy of pernicious anemia will remain injection of liver extracts. If allergy develops, folic acid under close supervision of the nervous system may be given; if neurological symptoms develop, therapy with vitamin B_{12} will probably be effective and harmless."

Uppsala, Sweden

64 WALDENSTRÖM J., and NORÉN B.: *Vitamin B_{12} (den antianämiska leverfaktorn) och allergi mot leverextrakt (Vitamin B_{12}, the anti-anemia factor in liver in cases of sensitivity to liver extract)* Särtryck ur Svenska Läkartidn. No. 3, 1949

Authors summary: "The possibility that the pure antianemic factor in liver extracts may be the allergen that causes reactions after prolonged injection therapy is discussed. If such cases could be found they might be regarded as excellent test objects for the detection of the presence or absence of the antianemic factor in different preparations.

"Eight patients with pernicious anemia who had been treated for long periods with liver injections, showed positive skin reactions when tested with several commercial liver extracts. Another patient was not only sensitive to liver but also to bovine protein. All nine patients showed no cutaneous reactions with pure vitamin B_{12} from Merck Co.

"It thus seems probable that allergic reactions are not usually caused by the liver factor and that the pure active substance may be used in future for the treatment of such allergic patients."

65. REISNER, E. H., JR.: *Treatment of pernicious anemia with crystalline vitamin B_{12}*, Am. J Med. 6 505, April 1949 (in Soc. Proc.)

West and Reisner treated 11 patients suffering from pernicious anemia in relapse with vitamin B_{12}. Maximal reticulocyte responses were obtained from a single injection of as little as 5 mcg.

The potency of the material as determined by these studies is slightly more than 1 International unit per microgram. Five of the 11 patients had combined system disease. The early cord lesions were completely relieved and later manifestations were greatly improved by the administration of vitamin B_{12}. Full restoration of the blood count occurred in patients maintained on this therapy The relationship between vitamin B_{12}, folic acid, and thymidine is not yet known.

66. SCHIEVE, J F., and RUNDLES, R. W: *Response of lingual manifestations of pernicious anemia to pteroylglutamic acid and vitamin B_{12}*, J Lab. & Clin. Med. 34 439-447 April 1949

The therapeutic effectiveness of synthetic pteroylglutamic acid in pernicious anemia is reviewed with particular reference to the lingual manifestations of the disease. In the authors cases, a few of which are reported in detail, lingual abnormalities have disappeared completely in some instances with administration of pteroylglutamic acid, and in others such treatment has had little effect. Two patients developed severe lingual relapses while taking 30 and 50 mg. of pteroylglutamic acid daily in spite of satisfactory hematologic and neurologic responses. In these 2 cases soreness of the tongue disappeared and papillary regeneration occurred within a week following a single intramuscular injection of vitamin B_{12}, 0.025 mg. in one case and 0.050 mg. in the other In 5 previously untreated cases of pernicious anemia with lingual manifestations a similar response was observed following administration of 0.001 mg. of the vitamin daily or 0.010 mg. in a single injection.

Duke University School of Medicine
Durham, N C.

67 WEST R., and REISNER, E. H., JR. *Treatment of pernicious anemia with crystalline vitamin B_{12}* Am. J Med. 6: 643-650 May 1949

Eleven cases of pernicious anemia, including the 3 cases previously reported (Abstr 33) have been treated parenterally with crystalline vitamin B_{12}. All have shown hematologic improvement, with blood counts rising to normal levels when weekly doses of 25 mcg. or less were given. The minimum effective dose has been found to be approximately 1 mcg. a day intramuscularly Five of these patients had combined system disease; none of them became worse, as has sometimes occurred during folic acid treatment, and all are now able to walk. Improvement varied in degree in the different cases. Reports of the 11 cases are given The current status of knowledge regarding the relationship of thymine, folic acid, cobalt, and vitamin B_{12} is discussed. Cobalt salts were given to one of the patients in this series without effect. Vitamin B_{12} appears to be the erythrocyte maturation factor of liver

Columbia University and
Bellevue Hospital
New York, N Y

68. WEST R. *The treatment of addisonian pernicious anemia with vitamin B_{12}* A.M.A. Scientific Assembly Official Program, June 1949 p. 95. (*See also* Abstr 67)

"Eleven patients with Addisonian pernicious anemia treated parenterally with vitamin B_{12} all showed a return of all the elements of the blood count to normal or slightly higher than normal levels. Five patients with spinal cord lesions of less than six months duration showed improvement of the cord lesions, including a return to normal of the Babinski reflex in the 1 case in which this reflex was abnormal. The minimal dose producing a maximal reticulocyte and blood response was 1 microgram a day by injection, smaller doses giving less than maximal responses. The effective oral dose appears to be considerably larger than the parenteral dose. Several cases of aplastic anemia, both idiopathic and secondary to drugs, have shown no response."

New York, N Y

69 JIMÉNEZ DE ASÚA, F: *La vitamina B_{12} en el tratamiento de la anemia perniciosa (Vitamin B_{12} in the treatment of pernicious anemia)* Rev Soc. argent. de hemat. y hemoterap. 1: 65-77 June 1949

The author presents a review of vitamin B_{12} together with reports of 4 cases of pernicious anemia treated with this agent. The results agreed with those obtained elsewhere. Great activity was shown by small quantities of vitamin B_{12}: in one patient who received a total of 50 mcg. in a little over a month, the erythrocyte curve was comparable to that produced in 4 other patients by 25 mg. of folic acid a day by mouth. The author believes that if larger doses were used—perhaps tenths of milligrams instead of micrograms—more lasting results would be obtained. Future treatment may consist of vitamin B_{12} with pteroylglutamic acid.

Hematologic Service of Camara College
Buenos Aires, Argentina

70. PINEY A., and BARKLEY T Y: *Sensitivity to liver* Lancet 1 1119 June 25, 1949 (in Letters to the Editor)

A patient with pernicious anemia became hypersensitive to liver extract after receiving it parenterally for about four years. Proteolyzed liver by mouth was substituted, but this did not have so satisfactory a hematologic effect, and it became extremely distasteful to the patient after several months. Anthisan (Neo-Antergan) did not prevent the allergic reaction to parenteral liver when an attempt was made to resume such treatment. An injection of 20 mcg. vitamin B_{12} and three further injections of this material (amount not stated) caused no untoward reactions. The patient's general condition improved and slight neurologic abnormalities almost disappeared.

71 WALDENSTRÖM, J., and NORÉN, B.: *Vitamin B_{12}, den antianämiska leverfaktorn och allergi mot leverextrakt (Allergy to liver extracts and vitamin B_{12}, the antianemic factor of liver)* Svenska läk. sällsk. förhandl. 46 157 160, Fasc. 3 1949 (abstr Quart. Rev Allergy 3 87 June-Sept. 1949)

Nine patients who were allergic to commercial liver extracts were tested with vitamin B_{12} intracutaneously None had a positive reaction. The test dose of vitamin B_{12} was about 1/100 of a full therapeutic dose (0.015 mg.) but much greater than the test doses of the commercial liver extracts. These results seem to confirm the view that allergy to liver extract is not attributable to the antianemic factor itself.

University of Uppsala
Uppsala, Sweden

72. KAUFMANN J., and COOPERBERG, A.: *Vitamin B_{12} in pernicious anaemia*, Canad. M. A. J 60 552-554, June 1949

Two patients with pernicious anemia were successfully treated with small doses of vitamin B_{12} given at repeated intervals. The dose given at each injection is not specifically mentioned, although the initial dose in one patient was 15 mcg., and in the other 25 mcg. The average daily dose thereafter was scarcely over 1 mcg. The reticulocyte and hematologic response of these patients to vitamin B_{12} was as adequate as could be obtained with the most potent liver extract.

The first patient had considerable central nervous system involvement, and the neurologic symptoms appeared to become aggravated after vitamin B_{12} was started and during the period of hematologic improvement, but disappeared with continued treatment.

73. CHALMERS, J N. M. *Sensitivity to liver* Lancet 2 88, July 9 1949 (in Letters to the Editor)

The writer used vitamin B_{12} derived from Streptomyces griseus (Glaxo Laboratories) in 2 patients with pernicious anemia who experienced severe reactions to liver extract given intramuscularly Both patients, who have objective manifestations of subacute combined degeneration of the cord, showed a good hematologic response, and their neurologic symptoms also seem to have improved. Repeated intramuscular injections of 12 mcg. of the B_{12} preparation have caused no constitutional disturbances or allergic reactions.

St. George's Hospital
London, England

74 REISNER, E. H., JR.: *The present status of vitamin B_{12} in pernicious anemia*, Bull. New York Acad. Med. 25 429-433 July 1949

This paper is largely a review of vitamin B_{12} clinical studies. The experience with patients in the First and Fourth Medical Divisions of Bellevue Hospital is summarized as follows "It was found that a single injection of as little as 3 to 6 micrograms produced a maximal reticulocyte response, and a total dose of 56 micrograms in one patient effected complete hematologic remission, which was maintained for four months before relapse, with no additional therapy A dose of one microgram a day was found to give a maximal reticulocyte response and restore the blood count to normal. A daily injection of 1/10 of a microgram was ineffective, but remission occurred when this was increased to ¼ of a microgram daily One-half a microgram daily gave a submaximal reticulocyte response. From these experiments the unit potency of B_{12} was fixed at approximately one unit per microgram, a unit being defined as the amount of antianemic substance required daily to effect and maintain hematologic remission in a patient with pernicious anemia. One of the Bellevue patients had exhibited early cord lesions at the onset of treatment with one gamma a day and at the end of fifty-three days of treatment had a negative neurological examination."

Bellevue Hospital
New York, N Y

75 MEYER, L. M., RITZ, N D., ROWEN M., BOCK, G., and RUTZKY J: *Treatment of pernicious anemia with animal protein factor concentrates of bacterial origin*, Bull. New York Acad. Med. 25 464, July 1949 (in Soc. Proc.)

A.P.F 60 and Normocytin, two animal protein factor concentrates prepared from the aerobic fermentation of a bacillus isolated from chicken feces, were tested for antipernicious anemia effect in patients with pernicious anemia in relapse. By chick assay A.P.F 60 had biologic activity equal to "10 unit liver" per cc. and Normocytin a potency of 10 mcg. of vitamin B_{12} per cc. By Lactobacillus leichmannii assay their respective potencies were 65 per cent of "10 unit liver" and 8 mcg. of vitamin B_{12} per cc. A.P.F 60 induced a satisfactory reticulocyte response in 4 of 5 patients and an increase of hemoglobin and red blood cells in all 5. Normocytin had these three effects in all of 8 patients, normal hematologic values being reached in 6, including one patient who previously had received A.P.F 60. All the patients experienced a feeling of general well-being, megaloblastic marrows were converted to normal, neurologic changes when present were improved, and there was no instance of progression or development of nervous system signs or symptoms.

76. BORTZ, D W: *The value of vitamin B_{12} in pernicious anemia preliminary report*, Cleveland Clin. Quart. 16 148-153, July 1949

The first 5 cases of pernicious anemia treated with vitamin B_{12} at the Cleveland Clinic are reported. All the

patients experienced a sense of well-being soon after this treatment was begun—one of them within 12 hours. The dosage varied widely 10 or 25 mcg. being injected at intervals of one day to one month. At the time of this report an attempt was being made to maintain the patients on a dose of 10 mcg. at two-week and four week intervals. Under vitamin B_{12} therapy the red blood count and hemoglobin rapidly return to normal, but there is a lag in the fall of the volume index to normal levels. Leukopenia, when present, clears up slowly

An addendum states that 3 additional patients with pernicious anemia have been placed on vitamin B_{12} therapy One with severe pyramidal tract involvement has responded well to intensive treatment. In the second, normal hematologic values have not been obtained, and coexistent liver disease is suspected. The third patient, who has carcinoma of the prostate, is responding satisfactorily

Cleveland Clinic
Cleveland, Ohio

77 ERF L. A., and WIMER, B. *Comparison of vitamin B_{12} from liver and from* Streptomyces griseus *in the treatment of pernicious anemia,* Blood 4 845-862, July 1949

A patient with pernicious anemia in relapse responded hematologically and clinically to two injections on successive days of 25 mcg. each of vitamin B_{12} from liver The case was followed for 114 days. The erythrocyte peak (3,900,000 cells/cu. mm.) occurred on the 86th day Two other patients with pernicious anemia in relapse who were treated with vitamin B_{12} derived from Streptomyces griseus experienced hematologic and clinical responses similar to those obtained by other investigators with vitamin B_{12} from liver in dosages of the same magnitude. One of these two patients had no neurologic complaints and received a single injection of 32 mcg. the other had severe subacute combined degeneration and was given two doses of 32 mcg. each, nine days apart. Definite neurologic improvements was seen in the latter case. Results in these 3 cases indicate that the factor from liver and that from Streptomyces griseus are closely related or identical.

Four of 5 patients with pernicious anemia in remission who had been receiving liver extract regularly reported subjective improvement following a single intramuscular dose of 32 mcg. of vitamin B_{12} from Streptomyces griseus, and 1 of the 4 showed a significant rise in the erythrocyte count.

The authors, in an addendum to their article, present further observations on these 8 patients and data on 8 additional cases of pernicious anemia in which vitamin B_{12} from Streptomyces griseus was administered. Some of the initial 8 patients were subsequently given additional vitamin B_{12} by injection or sublingually others were placed on liver therapy, and some required no further treatment at the time of writing. Allowing for individual variations, it is estimated that 50 to 100 mcg of the vitamin given by injection will produce a remission in pernicious anemia lasting for 50 to 100 days, and that about 50 mcg. given during the remission will maintain it for 70 to 120 days. General and neurologic complaints appear more difficult to control than the anemia. However glossitis consistently responds to treatment. That vitamin B_{12} therapy occasionally results in iron deficiency as remission occurs was exemplified in one case the deficiency was overcome by intravenously administered iron.

The results of sublingual therapy were inconclusive since the patients were in partial remission when this treatment was given. Slight reticulocytosis occurred in 2 of 3 trials of sublingual therapy (dosage 50 or 200 mcg.) and there was definite clinical improvement in each case.

78. BETHELL, F H., SWENDSEID M. E., MEYERS M. C., NELIGH, R. B., and RICHARDS H. G *Observations on the hemopoietic factors in hog stomach and duodenum, and the treatment of pernicious anemia by orally administered vitamin B_{12} in combination with extracts of duodenal mucosa,* Univ Hosp. Bull., Ann Arbor 15 49-53, July 1949

A review of the literature is presented, showing that vitamin B_{12}, in dosages sufficient to produce an optimal therapeutic response when administered parenterally to patients with pernicious anemia in relapse, is essentially ineffective when given by mouth. Orally administered vitamin B_{12}, however has proved effective when given in combination with human gastric juice, which indicates that vitamin B_{12} and Castle's extrinsic factor are either identical or closely related substances.

Since desiccated hog stomach and duodenum given orally are effective in pernicious anemia, it is considered that their activity might be due to a combination of vitamin B_{12} and intrinsic factor Microbiologic assay demonstrated in desiccated hog stomach a considerable amount of vitamin B_{12} which was not destroyed by heat. The vitamin B_{12} content was also confirmed by clinical response in 2 patients with previously untreated pernicious anemia.

Further clinical trials were performed using desiccated extracts of hog duodenal mucosa. One patient was first treated ineffectively with 5 mcg. of crystalline vitamin B_{12} a day given orally in two divided doses, and one with 10 Gm. a day of nondialyzable lyophilized duodenal mucosa extract containing both the soluble and insoluble fractions. In both cases a satisfactory hemopoietic response was obtained from the combined use of the two substances. A third patient failed to respond to 5 mcg. of vitamin B_{12} in the form of a concentrate given orally for six days, but responded to this treatment in combination with 1.5 Gm. of the lyophilized soluble portion of the nondialyzable duodenal mucosa extract.

These findings provide evidence that vitamin B_{12}, or a closely related substance, and intrinsic factor constitute the effective hemopoietic agents in hog gastric and duodenal tissue.

Ann Arbor Mich.

79 BEARD M. F., NATARO M., and LAYMAN L. H. *Vitamin B_{12} in pernicious anemia,* South. M. J 42 677-684, Aug. 1949

Six cases of pernicious anemia in relapse were treated with vitamin B_{12} intramuscularly with a view to determining the minimum dosage that will give an

mum hematologic response and observing the effect of this vitamin on the neurologic manifestations of pernicious anemia in relapse. All 6 patients showed hematologic improvement. The reticulocyte and erythrocyte responses were comparable to those observed in patients treated with liver and 1 microgram of vitamin B_{12} appeared to be approximately equivalent to 1 U.S.P unit of refined liver extract. To correct depletion of hemopoietic substance in the liver and bring about normal storage, an initial injection of 0.2 to 0.3 mg. of vitamin B_{12} followed by monthly injections of 0.03 mg. is suggested. In the authors cases, change from a megaloblastic to a normoblastic type of bone marrow was almost complete 48 to 72 hours after the administration of 0.025 or 0.05 mg. of vitamin B_{12}, and the change began six hours after the administration of 0.1 mg. in one patient. In 5 cases atrophy of lingual mucosa disappeared within four or five weeks and glossitis disappeared within 10 days the sixth patient did not have these manifestations. Minimum peripheral neuritis, present in 5 cases, improved moderately within four to six weeks. The combined sclerosis in 2 cases showed subjective improvement, but little change in the objective neurologic signs.

80. REISNER, E. H., JR., and WEST, R. *Effect of thymine desoxyriboside (thymidine) on human pernicious anemia*, Proc. Soc. Exper Biol. & Med. 71 651-652, Aug. 1949

Two patients with addisonian pernicious anemia in relapse were given single injections of 5.3 mg. and 150 mg. of thymidine, respectively The reticulocyte count rose, but not the red cell count. One week later daily intramuscular injections of 1 mcg. of vitamin B_{12} were begun, which produced a further rise in reticulocytes and an increase in the erythrocyte count. A third patient was given a suboptimal intramuscular dose of 0.25 mcg. of vitamin B_{12} daily The number of reticulocytes increased and then declined and the number of red blood cells increased. When 5 mg. of thymidine was given daily for nine days in addition to vitamin B_{12} there was a secondary increase in reticulocytes and red blood cells, and when thymidine was dropped the red blood cell count declined. It appears that thymidine is not completely inert hemopoietically but if it has an enhancing effect on B_{12} activity this is very slight. In view of the ability of other desoxyribosides to substitute for vitamin B_{12} in bacterial growth, the effect of mixtures of desoxyribosides on pernicious anemia should be investigated.

81. MEYER, L. M., RITZ, N D., CACCESE, A., RUTZKY J., SAWITSKY A., and BOCK, G *Studies in pernicious anemia patients treated with liver extract and folic acid antagonists* Am. J M. Sc. 218 197 203, Aug 1949

Five patients with pernicious anemia showed no hematologic responses when folic acid antagonists (pteroylaspartic acid and methyl pteroic acid) and adequate doses of liver extract were administered simultaneously This suggests that pteroylglutamic acid is necessary for the production of red blood cells. No clinical remission or reticulocyte response was obtained in a patient with pernicious anemia in relapse following intramuscular administration of 10 and 15 mcg. of vitamin B_{12} with 5 mg. of a-methopterin [sic] (4-amino-10-methyl pteroylglutamic acid) on the third and eleventh days of a 14-day course of treatment with a folic acid antagonist (methyl pteroic acid) the hemoglobin level and red and white blood cell counts did not rise, and sternal aspirations on four occasions revealed a megaloblastosis of 11 to 23 per cent.

82. SCHIEVE, J F., and RUNDLES, R. W *Response of lingual manifestations of pernicious anemia to pteroylglutamic acid and vitamin B_{12}*, Am. J Med. 7 256-257 Aug. 1949 (in Soc. Proc.)

Pteroylglutamic acid may fail to induce and maintain remissions of the lingual manifestations of pernicious anemia, as well as of the anemic and neurologic manifestations. Of 7 patients with pernicious anemia in relapse who had lingual mucosal atrophy, only 2 responded with regeneration of the filiform papillae in seven to ten days of treatment with 30 to 100 mg. of folic acid a day In the remaining 5, the lingual response was poor and 2 of them had definite lingual relapses during the third month of therapy When these 2 in relapse were given intramuscular injections of 0.010 and 0.025 mg. of vitamin B_{12}, the filiform papillae regenerated and the color of the tongue became normal in six and seven days, respectively In 5 patients with untreated pernicious anemia in relapse who also had lingual manifestations of the disease, regeneration of the papillae and restoration of a normal lingual color occurred six to ten days after treatment with a single dose of 0.001 mg. of vitamin B_{12} or with 0.0001 mg. daily

Duke University School of Medicine
Durham, N C.

83 JACOBSON B. M. *The treatment of pernicious anemia*, M. Clin. North America 29 1385-1400, Sept. 1949

This article contains a section in which the therapeutic efficacy of vitamin B_{12} is evaluated. A tabulation is presented of the effect of this vitamin on erythrocyte regeneration as reported in the literature by several investigators and as found in the author's own experience (4 cases) The author concludes that 1 mcg. of vitamin B_{12} is not equivalent to 1 U.S.P unit of liver extract, and that 2 mcg. a day does not result in a maximal hemopoietic effect nor equal the effect produced by 1.5 U.S.P units of liver extract administered daily

The use of commercial preparations of vitamin B complex containing small amounts of folic acid in the treatment of pernicious anemia is deprecated, for in early cases a small amount of folic acid may mask the early symptoms and early hematologic changes and render diagnosis difficult.

Harvard Medical School
Boston, Mass.

84 MARTIN, E., and VAUCHER, F : *Remarkable activity of proteolyzed liver extract in pernicious anemia*, Praxis 38 802- Sept. 15, 1949 (abstr. J.A.M.A. 142 446, Feb. 11, 1950)

The authors report on a liver extract which has undergone proteolysis under the influence of papain. This enzymatic action digests the proteins up to the polypeptide stage and makes it possible for the patient with pernicious

anemia to utilize Castle's intrinsic factor There is a solid and a liquid proteolyzed liver extract for oral use, as well as a highly concentrated proteolyzed extract for parenteral use. The latter was used in 15 patients with hyperchromic, macrocytic anemia In patients with per nicious anemia, 2 cc. of the extract are injected per week. In the preparation of this extract some of the vitamin B factors are lost, but the vitamin B_{12} content remains high. This proteolyzed extract is two to three times more potent than ordinary whole liver extracts.

85. KOHARI KUCHARIK, J *Reticulocyte response to liver therapy in pernicious anemia*, Lancet 2 1034 Dec. 3, 1949

86. UNGLEY C. C. *Vitamin B_{12} in pernicious anaemia parenteral administration*, Brit. M. J 2 1370-1377 Dec. 17 1949

Responses to 73 single doses of vitamin B_{12}, injected intramuscularly were studied in 53 patients with pernicious anemia in relapse. Care was taken to ensure that the responses were not due to dietary factors or sponta neous remissions, and a test was devised to measure the accuracy of the blood counts. The doses of vitamin B_{12} were graded logarithmically from 1.25 to 160 mcg. The reticulocyte response was compared, in 89 patients, with the expected response to liver extract (Isaacs and Friedman, *Am. J M Sc.* 196 718, 1938) but a better criterion of the response was the increase of red blood cells in 15 days.

By both measures of evaluation noted, a single dose of 10 mcg. of vitamin B_{12} produced as satisfactory a response as is usually obtained with liver extract. Larger doses produced an even greater increase of red blood cells in 15 days. The amount required to raise the red blood cell count above 4,500,000 per cu. mm. in 32 cases varied from 15 to 140 mcg. These amounts also led to an increase in white cells and platelets and to clinical recovery A maintenance dosage of 10 mcg. every two weeks was satisfactory in all but 3 of 21 patients followed for 6 to 15 months. The 3 exceptions were patients in whom soreness of the tongue was not relieved (one also had macrocy tosis) Six women developed iron deficiency after 8 to 15 weeks, which was relieved by ferrous sulfate. No new neurologic symptoms appeared, and those already present improved or remained unchanged. The results of vitamin B_{12} therapy in 8 patients with subacute combined degener ation—usually 40 mcg. weekly for at least 10 months—were as satisfactory as those obtained in a previous series of cases with crude or refined liver extract.

Ungley suggests a dosage schedule of 40 to 80 mcg. of vitamin B_{12} initially followed by 20 mcg. weekly for three months and 3 mcg. every three weeks thereafter for uncomplicated cases of pernicious anemia, and at least 40 mcg. weekly for six months followed by 20 mcg. weekly thereafter for patients with subacute combined degeneration. Some patients will need more, and at the least sign of relapse—hematologic, lingual, or neurologic—or if infection develops, the dosage should be temporarily doubled or trebled.

Royal Victoria Infirmary Newcastle-upon-Tyne, England

87 MILLS J., and HEMSTED E. H. *Treatment of pernicious anemia*, Lancet 2: 1151 Dec. 17 1949 (In Letters to the Editor)

All of 14 patients in their first attack of pernicious anemia showed a vigorous reticulocyte response to vitamin B_{12}, which was followed by a rapid return of hemoglobin and red cells to normal. Eighteen patients who had relapsed in spite of liver treatment were also successfully treated with the vitamin. A few patients under liver maintenance but not in relapse volunteered that they felt better when they changed to vitamin B_{12} therapy and generally their red cell levels rose from 4.0 to 4.5 million, to 5.0 million per cu. mm. Such consistent results have not been obtained with any of the liver extracts at present available in England.

Reading England

88. PAPPWORTH, M. H. *Vitamin B_{12} (from Streptomyces griseus) in pernicious anaemia*, Brit. M. J 1 1302 1303, June 3, 1950 (In Medical Memoranda)

Each of 2 patients with pernicious anemia was given an injection with 3 cc. of a concentrate from the metabolism fluid of a strain of Streptomyces griseus. An excellent response was obtained in both patients.

89 MEYER, L. M., RITZ, N D., ROWEN, M., BOCK, G., and RUTZKY J *Treatment of pernicious anemia with animal protein concentrates of bacterial origin*, Acta hemat. 3 305-322, June 1950.

90. HICKS, D G. T: *The treatment of pernicious anemia*, Post-Grad. M. J 29 305-307 June 1950.

91 NORÉN B. *Allergic reactions in parenteral liver therapy and vitamin B_{12}*, Acta med. Scandinav 137 48-65 Feb. 7 1950 (abstr Quart. Rev Allergy 4 80 June 1950)

Twenty four (18 per cent) of a series of 130 patients with pernicious anemia, treated with parenteral liver ex tract, manifested allergic reactions, the most common of which were itching and nausea. Only 5 patients had respiratory symptoms. On being tested with different com mercial liver preparations, 14 more of the 130 patients responded with positive skin reactions. Since they had no clinical symptoms, they have been classified by the author as "latent allergics." All of the 24 patients with clinical allergic symptoms to liver extract were able to tolerate vitamin B_{12} without any allergic reactions.

In an editorial note it is stated that this article is significant in that a large number of patients were carefully observed and vitamin B_{12} is proved to be nonaller genic. The classification of "latent allergics" is criticized, it being stated that the existence of latent allergy is problematic. Reference is made to the existence of a great number of persons who do not become sensitized despite repeated parenteral injections of liver extract known to sensitize others.

University Hospital Uppsala, Sweden

92. YOUNG W C., ULRICH, C. W., and FOUTS P J: *Sensitivity to vitamin B_{12} concentrate*, J.A.M.A. 143 893-894, July 8, 1950.

A patient with pernicious anemia was highly sensitive to liver extracts from both beef and pork liver A vitamin B_{12} concentrate made from streptomycin broth was given in doses equivalent to 5 mcg. of B_{12} every five days. After the sixth dose the patient complained of burning of the throat and itching and swelling at the injection site. Five or six more injections were given and similar discomfort was experienced each time. Medication was then changed to a vitamin B_{12} concentrate made from liver and 5 mcg. was given every five days for six months without untoward reaction. Because the supply of this concentrate was exhausted, a vitamin B_{12} concentrate made from streptomycin broth was again given. About 5 to 10 minutes after the patient received the eleventh injection, peripheral circulatory collapse developed. Sodium chloride infusions, epinephrine injections and oral administration of antihistaminic drugs brought about recovery in about three hours. Therapy was then changed to crystalline vitamin B_{12} made from streptomycin broth, and the patient has shown no evidence of sensitivity to this form of the vitamin.

Indianapolis General Hospital
Indianapolis, Ind.

93. SCOTT R. B.: *Diagnosis and treatment of pernicious anemia*, Brit. M. J 2 157 159 July 15, 1950.

A refresher course for general practitioners.

94 SCHRUMPF A. *B_{12} and folic acid in small doses in pernicious anemia*, Nord. med. 44 1197 July 28, 1950 (abstr J.A.M.A. 144 1039 Nov 18, 1950)

Six patients with pernicious anemia were treated with a combination of vitamin B_{12} and folic acid given orally in minimal doses. In 3 cases the daily dose was 10 mcg. of vitamin B_{12} and 0.67 mg. of folic acid in 2 cases the amount was doubled after 5 and 11 days respectively and in 1 patient the small dose was ineffective and liver extract was given parenterally followed by vitamin B_{12} and folic acid in increased doses. With successful vitamin B_{12} and folic acid therapy about 24 per cent of the hemoglobin deficit was covered, and a hemoglobin rise of 13.4 per cent every 10 days was attained. The reticulocyte reaction was later and less apparent than with parenteral therapy In all but one of the patients with leukopenia and thrombopenia, normal increase in leukocytes and thrombocytes occurred. Neurologic symptoms seemed to be relieved.

95 BORTZ, D W., and BATTLE, J D., JR.: *Massive use of vitamin B_{12} in treatment of pernicious anemia* Cleveland Clin. Quart. 17: 166-168, July 1950.

96. STRAUSS, M. B. *Vitamin B_{12} and pernicious anemia*, New England J Med. 243 187 194; 222 229 Aug. 3, and Aug. 10, 1950.

In this article are discussed and described the clinical aspects and pathogenesis of pernicious anemia, the value of vitamin B_{12} and folic acid in treating pernicious anemia, the possible relationship of vitamin B_{12} and the animal protein factor the relationship between vitamin B_{12} and growth factors for rats and other animals and the role of vitamin B_{12} in the synthesis of nucleic acid, and microbiologic assay methods for vitamin B_{12}. References on the effects of vitamin B_{12} in pernicious anemia and on other related macrocytic anemias are reviewed. Other physiologic effects of vitamin B_{12} in man are described, such as its effect on growth in children, and its diuretic effect. The relation of vitamin B_{12} to the extrinsic factor of Castle is mentioned. The importance of the differential diagnosis of pernicious anemia from other blood disorders is emphasized.

The treatment of pernicious anemia is outlined briefly During the first week of therapy 50 U.S.P units of liver extract or 50 mcg. of vitamin B_{12} is given. Thereafter 15 mcg. of vitamin B_{12} is given by injection once weekly until the blood values are normal. If there is considerable involvement of the nervous system, it is advisable to continue these weekly injections for the first year If the nervous system is not particularly involved, the weekly injections are followed by the administration of vitamin B_{12} every two weeks for the first year and then the frequency of injections is decreased to one in four weeks. The advantages of giving vitamin B_{12} by injection, rather than by mouth are mentioned.

Cushing VA Hospital
Framingham, Mass.

97 FULD H.: *Folic acid and pernicious anaemia*, Brit. M. J 2 527 Aug. 26, 1950 (In Correspondence)

The writer agrees with Dr Howard (*Brit. M J* 2: 417 Aug. 12, 1950) that folic acid per se is not the causal factor of neuropathy in cases of pernicious anemia treated with this agent. He does not, however agree that folic acid should be given in combination with liver extract or vitamin B_{12} in the treatment of pernicious anemia.

In addition, he reports a case of idiopathic steator rhea in which vitamin B_{12} was highly effective after folic acid had proved ineffective.

Liverpool, England

98. BEARD M. F., McILVANIE, S. K., and NATARO, M. *Vitamin B_{12} in pernicious anemia in remission*, South. M. J 43: 678-685, Aug. 1950.

Treatment of 18 patients with pernicious anemia who had been maintained on refined liver therapy (120 U.S.P units a month) was changed to 30 mcg. of vitamin B_{12} given once a month. Six of these patients had not responded completely to liver therapy but improved under B_{12} therapy 5 patients improved slightly or remained in the same condition after the shift in therapy; 7 (with one exception) who had been stabilized by liver therapy showed declines in hemoglobin levels, with or without subjective complaints, when they were maintained on vitamin B_{12} therapy One of these patients required 100 mcg. of vitamin B_{12} every two weeks before improvement became evident.

The results in these patients indicate that there is no definite correlation between the amount of liver extract and the amount of vitamin B_{12} required by the individual patient. In 11 of the 18 patients a dose of 30 mcg once a month was adequate but in the other 7 it was not. While vitamin B_{12} is a dramatic therapeutic agent for pernicious anemia in relapse, apparently it is not a complete replacement for liver extract as maintenance therapy in some patients.

In the discussion, Dr T D Spies stated that the B_{12} concentrates used by Dr Beard came from Streptomyces

and not from liver. While the B_{12} itself is the same in both concentrates, other substances may be present in the concentrate from liver which are effective in blood regeneration. The variabilities in source material, in methods of assay, and in the patients make variability in results inevitable. Dr. Spies thinks of raw liver as containing folic acid (to which some patients respond who do not respond to liver extract or B_{12}), vitamin B_{12} and other valuable nutrients. Oral vitamin B_{12} therapy is effective in some patients but not in all. The patient with pernicious anemia has enough vitamin B_{12} in the alimentary tract to be effective, but some help from liver or stomach preparations or gastric juices is required to get it through the gut wall.

University of Louisville School of Medicine and
Veterans Administration Hospital
Louisville, K.

99. VILTER, R. W., HORRIGAN, D., MUELLER, J. F., JARROLD, T., VILTER, C. F., HAWKINS, V., and SEAMAN, A. *Studies on the relationships of vitamin B_{12}, folic acid, thymine, uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias.* Blood 5: 695-717, Aug. 1950.

Authors' summary and conclusions: "Patients with pernicious anemia who are maintained on folic acid, 30 mg. three times a week, for two to three years may have a hematologic relapse which will remit satisfactorily if refined liver extract is given, or partially, if the dose of folic acid is increased to 50 mg. daily, or if thymine is given.

"The hematologic remission succeeding the increased dosage of folic acid is followed within several months by a second relapse. At this time the response of these patients to liver extract or vitamin B_{12} is retarded. Recovery occurs after two to four months.

"These experiments suggest that folic acid exerts its effect by mass action in pernicious anemia and that it is essential to the formation of thymine and other pyrimidines and purines or facilitates the utilization of these substances.

"Posterolateral column disease or peripheral neuritis occurred in every person with pernicious anemia who received increasing doses of folic acid to maintain an hematologic remission. This observation suggests that folic acid, by pushing a chemical reaction through to completion in the face of a serious deficiency of vitamin B_{12}, depleted the supply of this factor even more and led to the development of combined system disease.

"Uracil produced a hematologic response in 2 of 3 persons with pernicious anemia in relapse when given in doses of 15-30 grams daily. The data suggest that uracil may be a precursor of thymine.

"A patient with pernicious anemia of pregnancy failed to respond to uracil, 30 grams daily, but did respond partially when choline, 3 grams, and methionine, 6 grams, were given. Thymine induced a complete response. The partial response to methionine and choline and the better response to thymine suggest that choline and methionine supplied methyl groups for the formation of thymine, but that activating substances for the methylating process were missing.

"Reference is made to a patient previously reported from this laboratory who had liver extract and vitamin B_{12}-refractory megaloblastic anemia but who responded to folic acid and on a second relapse to thymine. Studies on the output of folic acid in the urine of this patient did not support the possibility of folic acid deficiency, and the suggestion was made that another substance, possibly the 'Wills factor,' was deficient, and that this factor together with folic acid activated the formation of thymine. These two factors correspond to the activators of the transmethylation reaction mentioned in the preceding paragraph.

"These studies on human beings and similar ones in bacterial metabolism suggest that folic acid, liver extract and vitamin B_{12} are essential to the formation of nucleic acid and nucleoprotein through a chemical chain reaction. The suggestion is made that the megaloblast common to pernicious anemia and related macrocytic anemias is a primitive erythroblast with an abnormality in the metabolism of nucleoprotein. The so-called maturation arrest in all marrow elements occurs because of this abnormality which may be induced by a deficiency of vitamin B_{12}, folic acid, the 'Wills factor,' and probably other chemical activators of this reaction."

University of Cincinnati
Cincinnati, Ohio

100. WILKINSON, J. F. *Vitamin B_{12}.* Lancet 2: 407, Sept. 23, 1950 (In Soc. Proc.)

"Dr. J. F. Wilkinson remarked that the dosage of B_{12} is steadily on the upgrade and he now gives 20-60 µg. weekly, but, even so, glossitis and poor counts persist in some cases. Allowance must be made for big variation in individual requirement of active material in patients with pernicious anaemia, hence only reasonably large series of cases are useful for estimating dosage. Dr. C. C. Ungley said he now gives a minimum of 60 µg. weekly, increasing up to 200 µg. if there are neurological complications."

101. BEARD, M. *Vitamin B_{12}.* Lancet 2: 407, Sept. 23, 1950 (In Soc. Proc.)

"Dr. M. Beard (U.S.A.) had a group of thirty-seven patients who were being treated with liver extract, not all satisfactorily. They were transferred to vitamin B_{12} treatment, 30 µg. monthly. At first they did well, the patients with low counts rising to normal levels. But after 7–11 months the counts fell and the M.C.V. rose again. Increase of B_{12} up to 100 µg. weekly had no effect; equally ineffective were supplements of folic acid, pyridoxine, iron, and ascorbic acid. Most of the patients had inadequate protein in their diet, and Dr. Beard thought that a factor in liver protein might be necessary for full recovery in pernicious anaemia."

102. STRAUSS, M. B. *Modern treatment of the anemias,* M. Clin. North America 34: 1291-1304, Sept. 1950 (Boston Edition)

Vitamin B_{12} and folic acid treatment for pernicious anemia is included in this review. The dosage schedule for vitamin B_{12} is given: initial administration is 50 mcg., then 15 mcg. is injected once weekly until the blood values return to normal. The injection is then given every two weeks for the remainder of the first year and thereafter once every four weeks. Advantages of injected vitamin B_{12} over oral administration are considered to be that the quantity required by injection is less, intestinal perme-

ability does not become a factor and treatment is kept under the control of the physician. Folic acid is not advised in pernicious anemia treatment since neurologic relapse often results, and may be made worse by increasing the dosage of folic acid. An impurity in the preparation may be responsible for these effects.

Cushing VA Hospital
Framingham, Mass.

103. UNGLEY C. C. *Vitamin B_{12} in pernicious anemia, Lancet 2: 468, Oct. 7 1950* (in Letters to the Editor)

In the report (*Lancet*, Sept. 23 [Abstr 100]) of the International Congress of Hematology Dr Ungley was quoted as saying that he now gives "a minimum of 60 µg. [of vitamin B_{12}] weekly increasing up to 200 µg. if there are neurological complications." In this letter he describes his current dosage practices. For routine purposes he uses the cheaper vitamin B_{12} concentrates, generally giving 60 mcg. every three weeks, with more frequent and perhaps larger doses (rarely more than 100 mcg. a week) for patients with subacute combined degeneration of the cord. He has 40 or 50 patients with little or no neurologic involvement who are receiving only 10 mcg. of vitamins B_{12} or B_{12a} every two weeks in a study comparing the effects of these two vitamins.

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

104 UNGLEY C. C.: *Absorption of vitamin B_{12} in pernicious anaemia. I Oral administration without a source of intrinsic factor* Brit. M. J 2 905-908, Oct. 21, 1950.

Author's summary "The absorption of vitamin B_{12} was studied by comparing the effective oral dose with the parenteral dose expected to produce a similar increase of red blood cells in 15 days. The apparent oral-dose/parenteral-dose ratio was corrected if the observed response to injected material was below expectation, indicating a relative resistance to vitamin B_{12}.

"In one case daily doses of 5 µg. gave no response. In another even 80 µg. a day for 24 days produced an increase in red blood cells no greater than would have been expected in 15 days from the injection of 2.5 µg. The response to injected material was normal, so that the corrected oral-dose/parenteral-dose ratio was several hundreds to one. By contrast the oral-dose/parenteral-dose ratio in five cases given a single dose of 3,000 µg. was from 20 to 40 1, the increase of red blood cells in 15 days being so great as to suggest the absorption of 80 to 160 µg. or even more. Presumably very little of the vitamin absorbed can have interacted with Castle's intrinsic factor considering the low quality and quantity of the secretion of the stomach in pernicious anaemia.

"Further tests are necessary to determine whether in fact more vitamin B_{12} is absorbed after a single large dose than after the same quantity of material given in divided doses daily"

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

105. ANGLESIO D *Nine cases of pernicious anemia treated with vitamin B_{12}*, Minerva med. 8- 802, 1950 (abstr Schweiz. med. Wchnschr 80 1125, Oct. 14, 1950)

Nine patients with pernicious anemia were treated with vitamin B_{12}. Improvement in the patients conditions was evident in 48 to 60 hours. The reticulocyte crisis occurred on the seventh day after which there was rapid improvement in the blood picture. The daily dosage of vitamin B_{12} was 2 or 3 mcg. and the total dose was 15 mcg. The treatment was repeated after a short interval.

106. QUERIES AND MINOR NOTES: *Pernicious anemia*, J.A.M.A. 150 1058, Nov 8, 1952.

Question "Please suggest treatment for a patient with true pernicious anemia in whom sensitivity to injections of vitamin B_{12}, in both the concentrate and crystalline forms, has developed. This is manifested by a generalized urticaria immediately following an injection. Preparations of vitamin B_{12} and folic acid for oral administration will not maintain her

Answer "Sensitivity reactions to vitamin B_{12} are rare. Since vitamin B_{12} is a physiological agent, it is more likely that the patient is sensitive to the vehicle in which vitamin B_{12} is dissolved, and it may be worthwhile to try products of several different manufacturers. The urticaria may also be checked by giving epinephrine (adrenalin®) in oil one hour prior to the injection of vitamin B_{12} or antihistaminics orally two hours prior to the injection. In some cases, 50 mg. of cortisone, given a few hours prior to administration of vitamin B_{12}, may be effective. Cortisone could probably be given without any danger of side-effects, since vitamin B_{12} would have to be administered only twice a month as a maintenance dose. Aside from that, desensitization to the injection of vitamin B_{12} preparations, similar to that used in liver extract therapy may be attempted. Very small amounts of the vitamin B_{12} preparation may be first injected intradermally and successive ones subcutaneously or intramuscularly Thereafter the final dose is repeated daily for three days and then weekly for six weeks, after which maintenance therapy with single injections every two weeks is given. In some cases, parenteral administration may have to be abandoned completely; in such cases, powdered stomach (ventriculin®) in doses of 30 gm. per day may be tried, with or without antihistaminics."

107 FLOOD F T., and LIMARZI, L. R. *Follow-up study of 310 cases of pernicious anemia*, J Lab. & Clin. Med. 36: 823-824, Nov 1950 (in Soc. Proc.)

Three hundred ten patients with pernicious anemia were followed in the hematology clinic for periods up to 17 years. Bizarre granulocytes appeared earlier and persisted longer after liver therapy was started. There were 5 relapses in patients on maintenance doses of liver Two of these patients were apparently receiving inactive liver since they recovered on the same dose of another preparation. Three were receiving liver at irregular intervals and recovered on recommended doses. One patient who showed a slow decline in red blood cells and hemoglobin on liver therapy gradually recovered on 15 mcg. of vitamin B_{12} weekly Three patients were sensitive to liver

To study the natural history of pernicious anemia, 43 patients without neurologic changes and with hemoglobin above 12 Gm. were given normal saline instead of liver and followed by periodic neurologic and hematologic examinations. Two patients developed cord changes after 17

and 26 months respectively; the remainder stayed in remission for 3 to 72 months—14 of them for more than 2 years. None of the patients had free hydrochloric acid on gastric analysis before or after prolonged and intensive liver therapy

Chicago, Ill.

108. MEYER, L. M., SAWITSKY, A., FINK, H., RITZ, N. D., and KRIM, M.: *Treatment of pernicious anemia with crystalline vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 75: 366-367 Nov 1950.

Four patients with pernicious anemia in relapse were treated with 1 mcg. of crystalline vitamin B_{12} daily for 10 days, and thereafter with 14 mcg. every 14 days. In all 4 cases the reticulocyte response was suboptimal and the blood count remained stationary in 3 cases and reached normal levels only after 108 days in the fourth.

A second similar group of 4 received 2 mcg. of vitamin B_{12} daily for 10 days, then 14 mcg. at weekly or biweekly intervals. All 4 showed satisfactory clinical improvement, optimal reticulocyte responses, and normal blood levels in 48 to 69 days. Persistent signs of neurologic disease were observed in all 4 cases, but the symptoms disappeared in 1 of them.

New York University College of Medicine
New York, N. Y.

109 MEYER, L. M., SAWITSKY A., COHEN, B. S., KRIM, M., and FADEM R.: *Oral treatment of pernicious anemia with vitamin B_{12}*, Am. J M. Sc. 220 604-609 Dec. 1950.

Seven cases of pernicious anemia in relapse were treated with vitamin B_{12}, given orally in daily doses ranging from 75 to 300 mcg. Five of the patients had a clinical remission and in 1 patient who had symptoms of neurologic disease prior to treatment these symptoms disappeared. Three of the 5 patients showed normal hematologic values In the remaining 2, they were subnormal. One of the latter patients had a severe concurrent pulmonary infection. This patient had a complete hematologic remission when treated with intramuscular vitamin B_{12}. One case had a maximal reticulocyte response, 3 had submaximal responses, and in the fifth patient no reticulocytosis occurred. The remaining 2 of the 7 patients did not respond to daily oral doses of 150 and 250 mcg. of vitamin B_{12}. They later responded to parenteral vitamin B_{12} therapy

In 1 patient, increasing the daily dose of B_{12} to 500 mcg. raised hemoglobin and erythrocytes considerably subsequent nausea and vomiting, however necessitated reduction of the dose. This side reaction was believed to be due to the large number of tablets and the amount of water ingested rather than to an effect of B_{12} itself.

It is concluded that there is apparently no clearcut universal oral dose of vitamin B_{12} for the treatment of pernicious anemia, that the deficiency of intrinsic factor in pernicious anemia patients is not always complete, and that a variable degree of absorption and utilization of B_{12} takes place in some cases.

Goldwater Memorial Hospital, and
Bronx Veterans Hospital
New York, N. Y.

110. LEAVELL, B. S., SHOTTON D., and CROCKETT C., JR.: *Pernicious anemia diagnosis and treatment*, Virginia M. Monthly 77: 635-642, Dec. 1950.

At the University of Virginia Hospital the incidence of pernicious anemia is 92 per 100,000 white admissions and 66 per 100,000 colored admissions.

Liver extract, folic acid and vitamin B_{12} are the most important preparations for the treatment of pernicious anemia. All of these agents produce a change from a megaloblastic erythropoiesis to the normoblastic type in about 72 hours.

Vitamin B_{12} is the most potent, weight for weight, of the three preparations. One microgram of vitamin B_{12} is about equivalent to one U.S.P unit of liver extract. Whether vitamin B_{12} equals or surpasses liver extract in the treatment of the neurological aspect of pernicious anemia remains to be established. Preliminary observations suggest that the vitamin is quite effective in this respect. In several patients with moderately severe neurologic involvement that have been treated with vitamin B_{12}, the authors noted that improvement seems to have been quicker and greater than is usually obtained with liver extract.

Folic acid is effective in producing a hematologic response in pernicious anemia and related macrocytic anemias, but it does not afford protection against the neurologic involvements of pernicious anemia. Its use in pernicious anemia is restricted to patients who develop hyper sensitivity to liver extract and to those who have no neurologic manifestations.

University of Virginia Medical School
Charlottesville, Va.

111 LECOQ R. *Mise en évidence de l'action synergique de l'acide folique et de la Cobamine (Vitamine B_{12}) dans la phase pseudo-irréversible de l'anémie pernicieuse (Evidence of the synergistic action of folic acid and Cobamine [vitamin B_{12}] in the pseudo-irreversible phase of pernicious anemia)* Thérapie 5 85-90, 1950.

Excellent experimental results have been obtained with B_{12} by English investigators in megaloblastic, gravid or puerperal anemia before the phase of pseudo-irreversibility When given too late, vitamin B_{12} has little effect, but is stored in the liver and when folic acid is administered later the reserves of vitamin B_{12} permit the manifestation of synergic action, and the folic acid exerts action together with vitamin B_{12}, which the latter could not exert alone.

In association with Charchard and Marone, the author has been able to demonstrate a terminal phase of pseudo-irreversibility which explains the clinical failure of folic acid and of vitamin B_{12}. It should not be forgotten that these two factors differ not only in the active doses but that they constitute two vitamin elements capable of replacing each other in early treatment of megaloblastic anemia; in the terminal phase they should be used together because they have a particularly efficacious synergism.

High doses of this combination may cause disturbances of the leukocyte component which adenine (vitamin B_4) corrects advantageously

112. KLIMA, R., and WENGRAF G.: *Effect of vitamin B_{12} in 34 cases of pernicious anemia*, Wien. med. Wchnschr 101 7 Jan. 6, 1951 (abstr J.A.M.A. 1301 1302, April 21, 1951)

Twenty-six patients with pernicious anemia were treated with pure vitamin B_{12}. A daily dose of 15 mcg. of vitamin B_{12} given intramuscularly or intravenously was adequate for restoration of a normal blood picture. Reactions of hypersensitivity or intolerance to vitamin B_{12} did not occur Mild symptoms of funicular myelitis, present in some patients were improved by vitamin B_{12}. Maintenance treatment with 15 mcg. per week of vitamin B_{12} was given to 8 patients for about 10 months after the anemia was corrected, and the improvement was maintained, 7 of the patients improving further Comparison of the results of vitamin B_{12} therapy with those of folic acid and liver extract therapy showed that with sufficiently high doses an optimum hematopoietic effect can be obtained with each of the three methods. Folic acid has the advantage over vitamin B_{12} in that it can be given orally in the same dosage as is required for parenteral administration. When vitamin B_{12} is given by mouth, 400 mcg are required to give the same effect as 15 mcg. given intramuscularly Combined preparations of vitamin B_{12}, folic acid and liver extract were given to 8 patients. These preparations did not seem to show mutual potentiation of effect. Vitamin B_{12} had a favorable effect on refractory nonregenerative anemia in some cases. Genuine aplastic anemia did not respond to vitamin B_{12}.

113 MILLS, J., and HEMSTED E. H. *Vitamin B_{12} in pernicious anaemia*, Lancet 1 237 238, Jan. 27 1951 (in Letters to the Editor)

Results are reported for a group of 144 pernicious anemia patients treated with vitamin B_{12} for a year The patients had previously been on controlled maintenance therapy with various liver extracts. Monthly intramuscular injections of vitamin B_{12} were given, 37 patients receiving 20 mcg., 49 receiving 40 mcg., and 58 receiving 80 mcg. per month. The highest average red cell levels occurred in the group given 80 mcg. per month; 98 per cent of these patients showed red cell levels above 4,500,000 per c.mm.

"When the condition of these patients treated with vitamin B_{12} is compared with their previous record under liver therapy it is found that 3% show slightly lower red-cell levels on vitamin B_{12} than on liver extract, 32% show no significant difference, and 65% are distinctly better on *vitamin B_{12} than they were on liver therapy* Thus a majority of our patients are distinctly better when treated with vitamin B_{12} than they were on liver therapy even though, as shown above, more than half the patients are receiving less than an optimal dose of vitamin B_{12}. In the group receiving 80γ of B_{12} each month, 80% show a higher red-cell level than they did on liver therapy

"From these observations, therefore, it would appear advisable to give at least 80γ of vitamin B_{12} at monthly intervals to maintain a red-cell level of 5,000,000 c.mm., and it is possible that a larger dose will give still better results. There is no doubt in our minds that these patients have been better in every respect since vitamin B_{12} replaced liver therapy"

Royal Berkshire Hospital
Reading, England

114 CHALMERS J N M. *Haemopoietic activity of vitamin B_{12a} and B_{12b} in pernicious anaemia*, Brit. M. J 1 161 164, Jan. 27 1951.

Nine cases of pernicious anemia in relapse were treated with crystalline vitamin B_{12}, and 5 cases with crystalline vitamin B_{12b}. Both substances were given intramuscularly in a single dose of 20 mcg. Both produced pronounced clinical improvement, with considerable relief of glossitis and lessening or disappearance of subjective neurologic symptoms. One patient, who had signs of associated iron deficiency showed progressive clinical improvement after a course of vitamin B_{12b}, given in a dosage of 40 mcg. every two weeks together with a course of oral ferrous sulfate. Normal blood values were attained in two months.

The author states that it is too early to draw conclusions however cases under investigation are showing *progressive clinical and hematologic improvement on* doses of 20 mcg. of vitamin B_{12b} given every two weeks.

St George's Hospital and Medical School
London, England

115 REID G C. K. *Two cases of pernicious anaemia treated with vitamin B_{12b}*, Brit. M. J 1 164-165, Jan. 27 1951

Two cases of pernicious anemia, 1 with early signs of subacute combined degeneration of the cord, were successfully treated with vitamin B_{12b} in the very small dosage of 10 mcg. In both cases the antianemic activity of B_{12b} was of the same order as that of crystalline vitamin B_{12}. In one case 10 mcg. of vitamin B_{12b} gave the same response as 1 cc. of examen, a liver extract having full antianemic activity at that dose level.

Walsall General Hospital
Staffordshire, England

116. EISENBERG S E. *The treatment of pernicious anemia*, Connecticut M. J 15 46-47 Jan. 1951.

Up to the present time, intramuscular administration of concentrated liver extract has been the treatment of choice for true addisonian pernicious anemia. *Now it* seems likely that vitamin B_{12} will take the place of liver extract. When administered intramuscularly 15 mcg. of vitamin B_{12} has the same effect as 15 units of liver extract. An average dose of 1 mcg. a day of vitamin B_{12} affects favorably the anemia, lesions of the mucous membranes, and the neurologic manifestations of pernicious anemia. It is the only pure chemical substance known which is effective in relieving subacute combined degeneration in persons with pernicious anemia.

Given orally vitamin B_{12} is ineffective unless normal gastric juice of stomach or intestinal extracts are also given. By this means, 5 mcg. a day has produced a therapeutic response.

Folic acid is contraindicated because it does not benefit the manifestations of subacute combined degeneration, and may even have an unfavorable effect on the neurologic manifestations.

New Britain, Conn.

117 HAUSMANN K. *Haemopoietic effect of thymidine in pernicious anaemia*, Lancet 1: 329-330, Feb. 10, 1951.

The author describes the treatment of 2 cases of addisonian pernicious anemia with a preparation made from purified liver extract and containing 85 to 90 per cent of thymidine. The average daily dose was 200 (100 to 250) mg The total dosages were 2 and 2.9 Gm., respectively These amounts exerted a hematopoietic effect comparable to that of corresponding doses of vitamin B_{12}, folic acid or thymine. The thymidine supply was exhausted after 16 days of treatment. The administration of vitamin B_{12} in doses of 25 and 50 mcg. produced no second reticulocytosis.

The author does not expect thymidine to be of practical value in the treatment of pernicious anemia. He states, however that the hematopoietic effect of thymidine supports the assumption that vitamin B_{12} exerts an enzymatic effect on nuclear metabolism.

Allgemeines Krankenhaus St. Georg
Hamburg, Germany

118. LICHTMAN H., GINSBERG V., and WATSON J : *Therapeutic effect of aureomycin in pernicious anemia*, Am. J Med. 10 239 Feb. 1951 (in Soc. Proc.)

In order to study the effect of alteration of the bacterial flora on pernicious anemia in relapse, preliminary studies were made on the effects of aureomycin in 3 cases of pernicious anemia and 1 case of nutritional macrocytic anemia. In the first case of pernicious anemia after a negative control period of 10 days on 3 mcg. daily of vitamin B_{12}, orally the addition of 3 Gm. of aureomycin daily resulted in a reticulocyte peak 17 days later The red blood cells, hemoglobin, and the hematocrit increased in 37 days. In the second case, after a negative control period of 12 days on a diet free of animal protein plus 2 Gm. of aureomycin a day the addition of 200 Gm. of chopped beef daily resulted in a hematologic response. In the third case, 2 Gm. of aureomycin and 2 Gm. of streptomycin orally each day resulted in a suboptimal but significant hematologic response.

The patient with nutritional macrocytic anemia was treated with a diet free of animal protein and 2 Gm. of aureomycin daily for 13 days with no appreciable effect when 3 mcg. of vitamin B_{12}, was given by mouth daily a reticulocytosis and rise of red cells occurred. In each case the sternal marrow which was originally megaloblastic, became normoblastic.

Kings County Hospital
Brooklyn, N. Y.

119 ELLISON R. R., WOLFE, S., LICHTMAN, H., GINSBERG V., and WATSON J : *Effect of citrovorum factor in pernicious anemia*, Proc. Soc. Exper Biol. & Med. 76 366-370 Feb. 1951

Citrovorum factor has been shown to produce adequate hematologic and clinical response in 4 of 6 patients with addisonian pernicious anemia and a submaximal response in another Treatment was ineffective in the sixth patient.

120. QUERIES AND MINOR NOTES *Folic acid and pernicious anemia*, J.A.M.A. 146 299 May 19 1951

Question "Does folic acid administered by muscle or by mouth predispose to cord damage in pernicious anemia? If folic acid and vitamin B_{12} are given together per os, is this danger averted? Would it be advisable to administer folic acid and vitamin B_{12} per os in patients with large subtotal gastrectomy in order to prevent development of pernicious anemia?

Answer "Folic acid whether administered orally or intramuscularly fails to protect against the development of cord damage and may predispose to the development of that condition in pernicious anemia. If vitamin B_{12} is administered orally in large enough doses it will protect against this complication, but it is much more effective given intramuscularly Neither folic acid nor vitamin B_{12} should be administered routinely in patients with large subtotal gastrectomy It would seem better practice for one to perform blood studies at suitable intervals to see whether a hyperchromic anemia was developing before instituting treatment."

121 CASTLE, W B. *Present status of the etiology of pernicious anemia*, Ann. Int. Med. 34 1093-1106, May 1951.

Author's summary "The information presently available suggests that Addisonian pernicious anemia is the clinical result of a deficiency chiefly of vitamin B_{12}. This deficiency is predominantly due to a lack of gastric secretion, itself usually a manifestation of hereditary predisposition and of advancing age. In some patients, local disease or surgical ablation destroys this essential function of the stomach. The consequent failure of vitamin B_{12} to be assimilated adversely affects hematopoiesis and frequently also the integrity of the alimentary tract and of the nervous system. The proximal cause of the macrocytic anemia is both decreased production and increased destruction of defective red cells. Either both processes must be of only moderate degree, or the magnitude of each must be inversely related to that of the other

Defective diets and intestinal dysfunction or disease, especially when gastric secretion is not significantly disturbed, may result in nutritional macrocytic anemia in which the predominant deficiency is not of vitamin B_{12} but of pteroylglutamic acid. Dietary deficiency of ascorbic acid probably restricts the formation of the metabolically active and closely related form of pteroylglutamic acid known as the citrovorum factor or folinic acid. Vitamin B_{12}, pteroylglutamic acid and ascorbic acid are apparently required for normal hematopoiesis. The full significance of their interrelations with respect to the nucleoprotein metabolism of microbes, of animals and of man remains to be disclosed."

Boston, Mass.

122. KLEINSORGE, H., and KLUMBIES G : *Vitamin B_{12} in treatment of pernicious anemia*, Med. Welt 20 740- June 2, 1951

123 JARROLD T., HORRIGAN D., THOMPSON C., and VILTER, R. W *The hematologic effect of folinic acid (citrovorum factor) in persons with pernicious anemia*, Science 113 688-689 June 15, 1951.

The authors cite studies from which the following deductions were made 1) folic acid is a precursor of the citrovorum factor and ascorbic acid may play a part in

the conversion; 2) citrovorum factor and folinic acid are probably identical 3) folinic acid may be a biologically important intermediate in the metabolism of folic acid; and 4) folinic acid may be the metabolically active form to which folic acid is converted in persons with pernicious anemia in relapse before it exerts its hematopoietic effect.

In the present study 3 patients with pernicious anemia in relapse were treated with folinic acid given intramuscularly for 10 consecutive days. The daily dose in 2 of the subjects was 3 mg. and in the other 1.5 mg. Hematologic responses occurred in all 3 patients. Results were as good as, but no better than, those which would be expected with similar amounts of folic acid. One of the patients who responded to the daily administration of 3 mg. of folinic acid had previously failed to respond to 0.6 mg. daily for 10 days.

An additional patient who had relapsed hematologically while receiving 20 mg. of folic acid daily did not respond to a 10-day course of citrovorum factor 3 mg. daily Subsequently he showed a desultory response to vitamin B_{12}, 15 mcg. daily for three weeks, then 20 mcg. daily

In another part of this study citrovorum factor was instilled directly into the bone marrow cavity of 3 patients with pernicious anemia in relapse in doses of 0.06 mg., 1.5 mg., and 3 mg., respectively In no instance was the erythrocyte maturation effect that follows similar administration of vitamin B_{12} observed. Folinic acid acts like folic acid in this respect.

The authors conclude from these results that folinic acid, or citrovorum factor is a potent hematopoietic agent in pernicious anemia in relapse but it is no more effective than a similar dose of folic acid. Moreover this agent, like folic acid, must be altered in the body in a site other than the bone marrow cavity before it becomes active in hemopoiesis.

University of Cincinnati
Cincinnati, Ohio

124 REISNER, E. H., JR., and WEINER, L.: *Observations on the mechanism of the synergistic action of oral vitamin B_{12} and folic acid in pernicious anemia*, Bull. New York Acad. Med. 27: 391, June 1951

Meyer and his associates attributed the synergistic action of vitamin B_{12} and folic acid when given together to an intrinsic factor-like action of folic acid, with enhanced absorption of vitamin B_{12}. Since folic acid is not the intrinsic factor of Castle, the authors sought some other explanation. Intrinsic factor does not affect absorption of vitamin B_{12} unless it is given within three hours of the latter but in 3 patients the authors found that 1 mg. or 0.67 mg. of folic acid increased absorption of 10 mcg. of vitamin B_{12} when given 12 hours after administration of B_{12}. In one instance, giving the two substances simultaneously following the initial response gave no secondary reticulocyte activity which suggests that the effect is not the direct action of one substance upon the other in the gut.

One patient was given 10 mcg. of vitamin B_{12} orally for a week, and 200 mcg. of folic acid parenterally for a week, and then both substances. The reticulocyte activity was increased when the drugs were given concurrently proving that synergy is not due to enhanced absorption of B_{12}. While the effect may be *additive*, the authors believe that it is due to "the catalytic action of minute amounts of B_{12} that are absorbed despite the absence of intrinsic factor upon the freely absorbable, but sub-optimal dose of folic acid." Further observation will be needed to see if the small amount of vitamin B_{12} is enough to prevent aggravation of spinal cord disease by the folic acid.

New York University Post-Graduate Medical School
New York, N. Y.

125 RITZ, N. D., MEYER, L. M., BRAHIN C., and SAWITSKY A.: *Further observations on the oral treatment of pernicious anemia with subminimal doses of folic acid and vitamin B_{12}*, Acta Haematol. 5 334-338, June 1951

A synergistic effect is noted when B_{12} and PGA are used.

126. JAMES, G. W., III, and ABBOTT L. D., JR.: *Effectiveness of thiocyanate analog of vitamin B_{12} in pernicious anemia*, Proc. Soc. Exper Biol. & Med. 77 416-418, July 1951

Vitamin B_{12} has been shown to be a cyano-cobalt coordination complex and has been designated cyanocobalamin. Vitamins B_{12a} and B_{12b} are identical, and represent the replacement of the cyano group by a hydroxy group in the vitamin B_{12} molecule. This substance is designated hydroxo-cobalamin. The thiocyanate analog, in which the cyanide ion of vitamin B_{12} is replaced by the thiocyanate ion, has been found to be microbiologically equivalent to vitamin B_{12} in the L. lactis cup assay but to differ from vitamin B_{12} in titrimetric assay methods.

The present paper reports studies of the action of the thiocyanate analog of vitamin B_{12} in 2 previously untreated patients with pernicious anemia in relapse. After an initial dose of 15 mcg., 5 mcg. was given daily parenterally until the peak of reticulocyte response was reached, after which 5 mcg. was given every other day In each patient, maximum reticulocytosis occurred on the seventh day of treatment. The moderately severe neurologic symptoms in the older patient (age 85) did not respond well. Treatment with 75 mcg. of the thiocyanate analog during nine days of hospitalization, and a total of 225 mcg. over a period of 61 days produced no noticeable improvement in the neurologic symptoms. Vitamin B_{12} was then given, since the supply of the thiocyanate was exhausted, and the patient reported some subjective improvement after 90 mcg. had been given. In both cases the early biochemical and hematologic responses were the same as those observed with the same dosage of vitamin B_{12}. Further evaluation of the neurologic response to the thiocyanate analog is necessary

Medical College of Virginia
Richmond, Va.

127 DAS GUPTA, C. R., and CHATTERJEA, J. B.: *Pernicious anemia in Asiatic Indians* Blood 6 631-638, July 1951.

In describing 3 cases of pernicious anemia in Asiatic Indians the authors corroborate the prevailing idea regarding the low incidence of pernicious anemia in this group.

One of the 3 patients was treated with folic acid, and during therapy neurologic complications appeared for the first time. This is typical in pernicious anemia treated with folic acid.

In their discussion, the authors state that pernicious anemia is apparently a pure deficiency disease in which a vitamin B_{12} deficiency is caused by a deficiency of intrinsic factor as a result of irreversible changes in the gastric mucosa. In nutritional macrocytic anemia, neurologic complications are absent because the presence of intrinsic factor assures the absorption of some vitamin B_{12} to preserve the integrity of the nervous system. Since folic acid completely cures nutritional macrocytic anemia, this disease may be a manifestation of folic acid deficiency The production by folic acid of an excellent hematologic response in pernicious anemia indicates that a disturbance of this vitamin may be an etiologic factor in this disease. In conclusion, it is stated that the respective roles of folic acid and vitamin B_{12} in the pathogenesis of pernicious anemia and nutritional macrocytic anemia, and also the relationship of one to the other are yet to be clarified.

School of Tropical Medicine, and
Carmichael Hospital for Tropical Diseases
Calcutta, India

128. CAMERON D G., TOWNSEND S. R., and MILLS, E. S. *Pernicious anemia. I Maintenance treatment with liver extract,* Canad. M. A. J 65 241 245, Sept. 1951.

This is a report of 63 patients treated for one to twenty years with liver extract. All but 8 (with complicating disease) were maintained in satisfactory hematologic remission.

Seven of fifty with objective neurologic disease at onset of observation underwent complete remission; no progression was noted, but rather improvement in the great majority of those with neurologic involvement.

Reports are presented for the purpose of comparison with a study in progress evaluating effect of B_{12} therapy on a long-term basis.

129 STRAUSS, M. B., and BROKAW R.: *Adrenocortical function in pernicious anemia,* New England J Med. 245: 798-802, Nov 22, 1951.

Manifestations common to both Addison s disease of the adrenal glands and pernicious anemia are listed and discussed in an article citing 50 references.

The authors made eosinophil counts before and after injection of corticotropin in 7 patients with pernicious anemia. In all, 53 counts were made and the fall in eosinophils was greater than 50 per cent in only eight of the tests. In contrast, all of 5 patients with hypochromic anemia due to blood loss showed significant falls in eosinophil counts following injection of corticotropin. Five patients with pernicious anemia in remission who had shown no significant declines in eosinophil levels after single doses of 25 mg. of corticotropin received 25 mg. every six hours for several days. Significant eosinopenia occurred in only 1 of the 5 patients. Three patients with pernicious anemia (who did not respond to corticotropin with eosinopenia) were given cortisone 100 mg. orally twice a day or every six hours for a few doses; all showed significant falls in eosinophil counts.

It has been observed that patients with pernicious anemia excrete below-normal amounts of 17-ketosteroids in the urine. The normal amount for males is 8 mg. of urinary 17-ketosteroids in 24 hours. Three male patients with pernicious anemia were studied and they excreted 2.4, 4.8, and 5.7 mg. of 17-ketosteroids in a 24-hour period before vitamin B_{12} therapy was instituted. The excretion was recorded as 3.2, 6.9 and 8.4 mg. respectively within two to three weeks after the initiation of vitamin B_{12} therapy The following comments concerning the possible relationship of vitamin B_{12} to adrenal function are made "It seems possible that a deficiency of this substance (vitamin B_{12}) leads to functional impairment of the adrenal cortex or the anterior lobe of the pituitary gland, either as a result of the general debilitating' effect of the deficiency or because vitamin B_{12} is actually necessary for their proper function. That the adrenal rather than the pituitary gland is involved is suggested by the failure of prolonged ACTH administration regularly to induce eosinopenia should it be determined that vitamin B_{12} is necessary for proper adrenal function, the possibility arises that a metabolic antagonist to vitamin B_{12} would be capable of inducing adrenocortical insufficiency The idea of producing such a state as a therapeutic procedure in serious hypertensive and other disorders by such means is intriguing If found to be beneficial it would presumably be much less hazardous than the present method of surgical extirpation of the adrenal glands."

Cushing VA Hospital
Framingham, Mass.

130. UNGLEY C. C. *Some current views on the origin of pernicious anemia and the absorption of vitamin B_{12}* Tr New York Acad. Sc. 14 25-28, Nov 1951

Royal Victoria Infirmary
Northumberland, England

131. CAMPBELL, D C., and HALL, B. E. *Maintenance therapy with vitamin B_{12} for patients with pernicious anemia,* J Lab. & Clin. Med. 38 797 Nov 1951 (in Soc. Proc.)

"Vitamin B_{12} has now been used for more than three years in the treatment of patients with pernicious anemia. Some question has been raised with regard to its ability to maintain these patients in satisfactory remission. Beard and his associates, in a preliminary study of eighteen patients with pernicious anemia maintained with vitamin B_{12} found the results to be unsatisfactory in seven patients. Mills and Hemsted, after following for one year 144 patients with pernicious anemia maintained on vitamin B_{12}, recommended a dose of 80 µg. per month.

"We have had the opportunity of following thirty two patients with pernicious anemia who have been maintained on vitamin B_{12} given intramuscularly for from twelve to twenty-nine months. All patients fulfilled the requirements for the diagnosis of pernicious anemia

including a histamine-fast achlorhydria. Fifteen of the group had evidence of subacute combined degeneration of the spinal cord, and eight additional patients had evidence of peripheral neuritis as indicated by paresthesia of the extremities. We have grouped these data regarding the maintenance dose of vitamin B_{12} both as to micrograms per day and as to total dose for the interval of administration. An attempt is made to correlate the patients clinical, hematologic, and neurologic status with these data."

Rochester Minn.

132. MEYER, L. M., and DIEFENBACH, W C. L. *Pernicious anemia treated with citrovorum factor (Leucovorum) report of a case*, Am. J Clin. Path. 21 1054-1056, Nov 1951.

Authors summary: "Oral administration for three months of 6.0 mg. of citrovorum factor to a patient with pernicious anemia induced a satisfactory remission, except that signs of subacute degeneration of the spinal cord appeared. Neurologic changes were reversed after this therapy was discontinued and vitamin B_{12} was administered parenterally"

Veterans Administration Hospital Bronx, N Y

133. REIMER, E. E.: *Clinical experiences with vitamin B_{12} therapy* Wien. klin. Wchnschr 63 941 Dec. 14, 1951 (abstr J.A.M.A. 148 1256, April 5 1952)

The abstract reads, in part: "Based on treatment of more than 100 patients with pernicious anemia with vitamin B_{12}, a dose of 120 to 180 µg. depending on the severity of the anemia and the associated neurological symptoms, may be required for recompensation. Several daily doses of 30 µg. seem to exert a better antianemic effect than one massive dose. In 80 patients subjected to continued treatment, a monthly maintenance dose of 40 µg (in divided doses of 20 µg. every two weeks) was required to obtain erythrocyte counts of 4,000,000 Several of these patients were treated with vitamin B_{12} for more than two years without requiring increasing doses, while increasing doses of folic acid were required for maintenance. The subjective well-being of all the patients was pronounced, and increases in weight and in mental alertness were observed frequently Larger maintenance doses up to two times the usual dose are indicated in elderly patients with hypoproteinemia or neurological manifestations. Fifteen patients with pernicious anemia of more than three years duration whose blood values decreased markedly following discontinuation of the liver maintenance dose were subjected to a therapeutic trial with orally administered tablets containing 5 µg. of vitamin B_{12}, 1 mg. of folic acid, and 0.25 gm. of extract of stomach mucous membrane. Four tablets were given daily for four months. Although this follow-up is too short for definite evaluation of the maintenance therapy three to four tablets stabilized the blood values and increased the red blood cell count to 4 000,000, which had never been reached previously The subjective well-being of the patients was remarkable, and neurological manifestations did not occur Fairly normal conditions of absorption are indispensable for oral treatment with vitamin B_{12}. Parenteral administration of vitamin B_{12} is recommended in patients with intercurrent infections, hypoproteinemia, severe diarrhea, and neurological manifestations. In 10 patients with decompensated pernicious anemia and associated mild to severe herpes zoster highly satisfactory results with disappearance of the cutaneous manifestations and the neuritic complaints were obtained with four or five massive doses of from 30 to 60 µg. of vitamin B_{12}.

134 PAUL, J T *Treatment of pernicious anemia*, Postgrad. Med. 10 508-510, Dec. 1951.

Treatment with liver extract and vitamin B_{12} is briefly discussed. Patients with neurologic lesions require higher doses than others. If the vitamin is given orally alone, 30 to 60 times the amount of the parenteral dose is necessary unless the vitamin is mixed with normal gastric juice.

University of Illinois College of Medicine Chicago, Ill.

135 LUDWIG L. *Untersuchungen zur bakteriellen synthese von Vit. B_{12} im menschlichen Magendarmkanal (Bacterial synthesis of vitamin B_{12} in the human gastrointestinal tract)* Klin. Wchnschr 45/46, 770- , 1951 (abstr Schweiz. med. Wchnschr. 82 732, July 12, 1952)

The apparent findings of the experiments seem to be that feces extracts from pernicious anemia patients have hematopoietic activity when tested microbiologically and when given parenterally to patients with pernicious anemia. The author concludes that intestinal bacteria are able to synthesize vitamin B_{12} in patients with pernicious anemia, but the presence of proteins in the diet is necessary for this.

136 GALT J., HUNTER, R. B., and HILL, J M. *Pernicious anemia superseded by polycythemia vera report of a case*, Am. J M. Sc. 223 61-64, Jan. 1952.

A case history is described in which a patient with pernicious anemia was treated with vitamin B_{12} and crude liver extract remission occurred. About a month after an acceptable hemoglobin level was reached the patient developed pain, swelling and redness of the right leg, which were diagnosed as symptoms of erysipelas. There was a slow but total remission on bed rest and penicillin therapy but it did not last. Six months after the remission from pernicious anemia it was apparent that the patient had polycythemia vera.

In discussing the unusual circumstance of a patient's having both pernicious anemia and polycythemia vera, the authors state "Speculation at the present time in regard to this unusual association of these diseases has little basis in known facts. It has been suggested, however that when they do co-exist, the basic stimulus for medullary over-production prevailing in polycythemia is present but that the nutrient factor involved in clinically established pernicious anemia would hinder any polycythemic response. Only upon administration of the missing anti-anemic factor could the polycythemia then emerge.

"One could surmise that excessive amounts of specific anti-anemic therapy might over-stimulate the marrow into producing an overt polycythemic state. Except for the occasionally dissimilar circumstance of a slightly over reactive erythrocytic response previously mentioned, there is nothing to substantiate this.

"Some investigators have theorized that primary polycythemia is the antithesis of pernicious anemia with the over production of cells being due to excessive formation of hematopoietic factor Experimental attempts to demonstrate this have not been convincing."

Baylor University Hospital
Dallas, Texas

137 GLASS, G B. J., BOYD L. J., RUBINSTEIN M. A., and SVIGALS C. S. *Treatment of pernicious anemia by oral administration of vitamin B_{12} and glandular mucoprotein recovered from gastric juice of humans* Am. J Med. 12 109 Jan. 1952 (In Soc. Proc.)

Eight patients with pernicious anemia in relapse were given 7 to 30 mcg. of vitamin B_{12} daily and after a control period 50 to 200 mg. of glandular mucoprotein obtained from gastric contents rich in this substance was added. The results varied, but the general improvement in response when mucoprotein was given suggests that Castle's intrinsic hematopoietic factor is contained in or is identical with the glandular mucoprotein of the human gastric juice.

New York Medical College,
Flower and Fifth Avenue Hospitals, and
Metropolitan Hospital Research Unit
New York, N Y

138. STURGIS, C. C. *Etiology and treatment of pernicious anemia*, Postgrad. Med. 11 83-89 Feb. 1952.

Development of red blood cells in the bone marrow is now believed to depend on two dietary constituents, namely vitamin B_{12} and folic acid. Vitamin B_{12} is absorbed from the stomach as a result of the action of the intrinsic factor of Castle in the gastric secretion, is then stored in the liver as the erythrocyte maturing factor and is released as needed to control the rate of maturation of erythrocytes in the bone marrow Folic acid is present in the food in the conjugated state. In the body it is split into free folic acid and changed by enzyme action into folinic acid, which also functions to control the rate of maturation of red blood cells. Pernicious anemia is thought to be caused by a deficiency in the intrinsic factor in the gastric juice which impairs the absorption of vitamin B_{12}, creating a deficiency in this vitamin. For a time, folic acid continues to control the maturation of the red blood cells, but eventually the increased demand for folic acid produces a deficiency in this vitamin also, which causes delayed maturation of erythrocytes. It would be anticipated that administration of either folic acid or vitamin B_{12} would be effective in controlling pernicious anemia. Usually the administration of vitamin B_{12} to pernicious anemia patients in relapse produces an increased appetite which results in an increased intake of folic acid in foods. In a few patients administration of vitamin B_{12} does not repair the deficiency of folic acid, and better results are obtained when folic acid is given in addition to vitamin B_{12}.

The bearing of this theory on the treatment of different types of macrocytic anemia is discussed. In general, macrocytic anemia will respond to folic acid in doses of 10 mg. daily but this is ineffective in the control of the neurologic manifestations of pernicious anemia, which respond to vitamin B_{12}. Practically all types of macrocytic anemia respond to vitamin B_{12}, with the exception of the macrocytic anemia of pregnancy in which folic acid is highly effective.

Three patients were presented to illustrate types of anemia and their treatment. One with pernicious anemia with neurologic manifestations had responded rapidly after a single intramuscular injection of 500 mcg. of vitamin B_{12}. Another had been maintained on liver therapy for 25 years. Neurologic involvement in this case had been present for only a few months when vitamin B_{12} was started, and improved under liver treatment except for ataxia resulting from permanent injury to the spinal cord. Since the sole effective therapeutic component of refined liver extract is probably vitamin B_{12}, this vitamin is recommended, beginning with 20 mcg. intramuscularly every day for a week, and then at greater intervals. If, as occasionally happens, the increase in red blood count levels off at about 4,000,000 per cc., 5 mg. of folic acid should be given daily by mouth until the count is normal (4,500,000 for women and 5,000,000 for men)

The third patient was presented to illustrate macrocytic anemia not due to pernicious anemia, and to show the points to be considered in diagnosis. This patient had cirrhosis of the liver The treatment recommended was folic acid with the addition, if this proved ineffective alone, of vitamin B_{12} intramuscularly

University of Michigan Medical School
Ann Arbor Mich.

139 SPRAY G H., and WITTS L. J *The utilization of folic acid given by mouth*, Clin. Sc. 11 273-281, Aug. 1952 (abstr J Clin. Nutrition 1: 260 March-April 1953)

The increase in folic acid concentration in the plasma and in urinary excretion of this vitamin after oral administration of 1 mg is much smaller in untreated pernicious anemia patients than in normal subjects. Treatment with liver extract or vitamin B_{12} restores the response to oral folic acid. These results suggest that absorption of folic acid is impaired in pernicious anemia in relapse or that its utilization is increased. The authors favor the latter suggestion. Shortage of vitamin B_{12} in pernicious anemia is supposed to increase the demand for folic acid.

140 PAVLOVSKY A.: *Foreign Newsletter—Argentina*, Blood 7 478-480 April 1952.

Included in this communication is the following reference to treatment of pernicious anemia "The same author has used vitamin B_{12} orally and rectally with success. Consequently he revises the present concept of the importance of the deficiency in intrinsic factor in the pathogenesis of pernicious anemia. De Arma has also confirmed the results of Meyer and his co-workers on the efficacy of the association of small doses of folic acid to vitamin B_{12}."

Buenos Aires, Argentina

141. TALLEY R. W., DOHERTY J E., and SHUKERS C. F: *Pernicious anemia complicated by acute granulocytic leukemia a case report*, South. M J 45 559-561, June 1952.

A patient who had been treated for pernicious anemia at intervals over four years developed acute granulocytic leukemia. At various times the pernicious anemia had responded to folic acid, to liver extracts, and to vitamin B_{12}. Neither liver extracts nor vitamin B_{12} gave an erythrocyte or reticulocyte response after leukemia developed, and the patient died in about two months.

Four cases in which pernicious anemia and granulocytic leukemia coexisted are reported from the literature. This coexistence is rare, and is probably a coincidence. However potent liver preparations affect the production of granulocytes as well as the production of erythrocytes, and the possible role of liver extracts, folic acid, and vitamin B_{12} in stimulating abnormal production of granulocytes should be investigated.

University of Arkansas School of Medicine
Little Rock, Ark.

142. REISNER, E. H., and WEINER, L. *Studies on the mutual effect of suboptimal oral doses of vitamin B_{12} and folic acid in pernicious anemia*, New England J Med. 247 15-17 July 3, 1952.

Results of a study of the interrelation of folic acid and vitamin B_{12} therapy in 4 patients with pernicious anemia are given. Folic acid was administered intramuscularly or orally in doses ranging from 1 to 0.15 mg. and vitamin B_{12} by the same methods in doses ranging from 0.25 to 30.0 mcg. One patient responded completely to a daily oral dose of 1 mg. of folic acid, and another responded to 30 mcg. of vitamin B_{12} given parenterally on two successive days, but not to parenteral folic acid (0.5 mg.) or oral folic acid (1 mg.) and B_{12} (10 mcg.) combined. These 2 cases illustrate the varied response of pernicious anemia patients to folic acid.

In the other 2 patients, oral administration of 10 mcg. vitamin B_{12}, separated by a 12 hour interval from administration of 0.67 to 1 mg. folic acid, resulted in enhanced hematopoietic activity over that achieved with either drug alone. The dosage of each drug was suboptimal. These cases confirm the work of Meyer (*Am. J Clin. Path.* 20 454-457 1950) who suggested that folic acid acts like "intrinsic factor" But since the enhanced response is also found when the agents are given at 12 hour intervals, the authors state "Consequently the enhanced response is not due to increased absorption of vitamin B_{12} from the gut, as is presumably the case with the intrinsic factor of gastric juice, but is probably the result of the additive or synergistic effects of the small amounts of B_{12} that may be absorbed by at least certain patients with pernicious anemia and the readily absorbed folic acid."

New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N Y

143 MURPHY W P: *Twenty five years experience in treatment and management of pernicious anemia*, J.A.M.A. 149 907 912, July 5, 1952.

The author traces the sequence of events that have gradually led to present-day recognition of signs and symptoms of pernicious anemia. Therapeutic agents, their specific uses and results of treatment are discussed.

In describing the work of the numerous investigators in the search for the active liver principle, the author refers to the research involving vitamin B_{12}

"Subsequent work has supported the belief that vitamin B_{12} is the factor or one of the factors of liver and its extracts that is effective in the treatment of pernicious anemia. The fact that it is effective when administered parenterally and is so only in much larger doses when ingested has led to the belief that it not only is the extrinsic factor of Castle but is actually the anti-pernicious-anemia factor Hall observed that the response to orally administered vitamin B_{12} in doses up to 100 times the minimal effective dose for parenteral administration was variable and unpredictable but that its effectiveness might be increased by providing a known source of intrinsic factor such as the gastric juice of normal human beings and extracts of the stomach and intestines of swine. The efficacy of ingested whole mammalian liver as a hemopoietic in megaloblastic anemias and for control of the central nervous system degeneration in those with associated achlorhydria is well known. Liver contains both folic acid and vitamin B_{12}. The quantity of vitamin B_{12} present in therapeutically effective amounts of liver is less than the amount of the crystalline form that is effective when administered by the oral route. The quantity of folic acid present is also small, and, although it may be effective for hemopoiesis when ingested, it does not control the central nervous system changes. Meyer and his associates reported favorable results from the oral administration of vitamin B_{12} combined with minimal amounts of folic acid. These and other data accumulating suggest that there is a cooperative action between folic acid (and/or its conjugates) and vitamin B_{12} for the control of hemopoiesis in megaloblastic anemia and of central nervous system function in the presence of achlorhydria. Vitamin B_{12} may act within the body to convert folic acid conjugates to folic acid so that they become available for hemopoiesis or it may enhance the utilization of folic acid for the protein metabolism of blood formation. In view of the rapidly accumulating knowledge of the mechanism of the effect of liver in the megaloblastic anemias, it may be desirable to revise the present concept of the nature of the intrinsic factor or the role it plays in the utilization of the anti-pernicious-anemia factor"

Included in the author's comment are the following references to achlorhydria: " Histamine-fast achlorhydria is the result of a congenital defect of the mucosa of the stomach and is probably present from birth in the person who at some time, presents other evidences of the disease. The well-recognized familial incidence of pernicious anemia should make it possible to anticipate the disease in other members of the families of these patients and to start preventive therapy in those with achlorhydria. The physician should be alert to the earliest signs and symptoms in all patients who present themselves for study

"The recent demonstration by Segal and his associates of a method for determining the presence or absence of free hydrochloric acid in the stomach by means of an

ion-exchange resin, in place of the usual unpleasant method of gastric analysis, should simplify the problem of diagnosis. As both the physician and layman become more acutely aware of the benefits to be derived from liver and its extracts, early recognition and treatment should become practically universal."

The review is accompanied by 35 references.

36 Gloucester St.
Boston, Mass.

144 BLACKBURN E. K., BURKE, J., ROSEMAN C., and WAYNE, E. J: *Comparison of liver extract and vitamin B_{12} (cyanocobalamin) in maintenance treatment of pernicious anaemia*, Brit. M J 2 245-248, Aug. 2, 1952.

Authors summary "Sixty patients suffering from pernicious anaemia have been maintained in good health on injections of vitamin B_{12} (cyanocobalamin)

"Of these, 22 had been previously treated with liver extracts for relatively long periods and were then given vitamin B_{12} for at least two years. Only one case showed a statistically significant decrease in the red-cell count 11 cases had a significant increase. No case showed a significant decrease in haemoglobin concentration.

"The first 10 of the patients who had been treated with vitamin B_{12} from the start of their illness showed significantly higher red-cell counts and haemoglobin concentrations when compared with 10 patients who received liver extract alone.

"A few patients were strikingly better on vitamin B_{12} treatment; one relapsed because of too infrequent dosage.

"No evidence was found of sore tongue, gastrointestinal disturbance, neurological involvement, or leucopenia. Macrocytosis may persist.

"Vitamin B_{12} injections are small in bulk, constant in potency relatively cheap, and do not lead to sensitization. A maintenance dose of at least 50 μg. a fortnight is recommended for all patients with pernicious anaemia without neurological involvement."

University of Sheffield, and
Royal Infirmary and Hospital
Sheffield, England

145. BREWERTON, D A., and ASHER, R. A. J *The maintenance therapy of pernicious anaemia with vitamin B_{12}*, Lancet, 2 265-266 Aug. 9 1952.

This is a report on maintenance therapy of pernicious anemia with vitamin B_{12} in 36 patients. Each has received at least 20 months therapy with vitamin B_{12} and had previously been treated with liver preparations for at least a year Dosage of vitamin B_{12} ranged between 50 mcg. every four weeks to 100 mcg. every two weeks.

Subjectively 20 patients reported they felt better on vitamin B_{12} than they had on liver 13 felt no difference, and 3 felt worse. Of these latter one has a senile tremor which has been becoming more severe for five years one has had severe subacute combined degeneration of the cord for eight years and her recent symptoms were relieved when she was given twice as much vitamin B_{12} as she had been receiving and the third was found on examination to be as well objectively as before, and review of old records showed the same complaints had recurred many times in the past 10 years while she was receiving liver injections.

Clinical examination at the time of subjective evaluation showed no evidence of deterioration. In no case was the red-cell count below 4,000,000 per cubic millimeter for women or 4 400,000 for men. In commenting on vitamin B_{12} the authors state: "It is cheap, relatively painless, and free from unpleasant reactions. Its potency is reliable."

Central Middlesex Hospital
London, England

146. REISNER, E. H., JR., and WEINER, L.: *Treatment of pernicious anemia with massive parenteral doses of vitamin B_{12}*, Bull. New York Acad. Med. 28 Aug. 1952 (in Soc. Proc.)

Urinary excretion studies on patients receiving injections of 100 to 1,000 mcg. of vitamin B_{12} showed that 51 to 98 per cent of the injected dose could be recovered from the urine in the succeeding 72 hours. This seems to indicate that parenteral doses of more than 50 mcg. are largely wasted and therefore of no advantage over the smaller doses in the treatment of pernicious anemia. In 14 patients with pernicious anemia in relapse who were treated at weekly intervals with single injections of 1,000 mcg. of vitamin B_{12} remissions of three to six months occurred in 7 and 4 had been in remission four six, eight, and nine months when they were lost sight of. One patient did not show complete remission following the injection and developed an exacerbation of cord disease two months later In 7 patients with chronic combined system disease due to pernicious anemia, weekly injections of 1,000 mcg. of vitamin B_{12} for periods of 4 to 18 weeks gave no improvement greater than would have been expected from conventional doses of vitamin B_{12} or liver extract.

New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N Y

147 WALKER, W., and HUNTER, R. B. *Single massive dose of vitamin B_{12} in untreated pernicious anaemia*, Brit. M. J 2 593-595, Sept. 13, 1952.

Fifteen patients with untreated pernicious anemia were given single 1 mg. intramuscular doses of vitamin B_{12} and their progress was followed until relapse no other antianemia therapy was given, except for oral iron when indicated. The clinical state and marrow closely corresponded with the blood counts, except that only one patient complained of lassitude when the red cell count fell below 4,500,000 (the criterion for relapse) The other patients still felt normally well at relapse.

Six of the patients have continued in remission after 84 to 358 days since their single injection of vitamin B_{12}. Seven of the patients who relapsed were given a second similar dose of vitamin B_{12}; 3 are still in remission six to twelve weeks later and the other relapsed 142 to 349 days after the second injection.

Neurologic symptoms disappeared, when they had existed, in all but one patient. This patient had paresthesia at the time of relapse following the first injection, condition disappeared after the second

The authors state in their summary: "While of theoretical and practical interest, these findings do not justify without further trials, the routine treatment of pernicious anaemia by 1 mg. doses of vitamin B_{12} at long intervals."

St. Andrews University and Maryfield Hospital Dundee Scotland

148. CONLEY, C. L., GREEN T W., HARTMANN R. C., and KREVANS, J R. *Prolonged treatment of pernicious anemia with vitamin B_{12}*, Am. J Med. 13 284-293, Sept. 1952.

Results of vitamin B_{12} treatment of more than 100 patients with pernicious anemia are discussed.

Seven patients were given single intramuscular injections of 12.5, 25 or 100 mcg. of vitamin B_{12} with no further therapy until the hematocrit stopped rising. In some cases the lowest dose produced a striking response, but 100 mcg. seemed to be necessary to produce a maximal hematologic effect. The general state of health usually improved within the first week. Neurologic response was variable. A single dose of 25 mcg. seemed to be more effective than 1 mcg a day for 25 days.

Fifty four patients have been successfully maintained on a regular dosage schedule for more than one year; in 48 cases 45 mcg. has been given intramuscularly every six weeks, 2 patients have been given 150 mcg. every five months, 1 has received 150 mcg. every four months, 1 has received 30 mcg. every four weeks, and 2 have received 150 mcg. every four weeks.

No evidence has been found to support the theory that folic acid deficiency in addition to vitamin B_{12} deficiency may exist in patients with pernicious anemia. All the patients in this series did well on vitamin B_{12} alone. Administration of folic acid brings about a hematopoietic response but may permit development of severe subacute combined degeneration before adequate vitamin B_{12} treatment is given. Ingestion of folic acid in multi vitamin preparations has sometimes resulted in the confusing picture of subacute combined degeneration in the absence of anemia. The authors state that folic acid has no place in the treatment of uncomplicated pernicious anemia although it does no harm in patients adequately treated with vitamin B_{12}, it is unnecessary in patients on normal diet.

Vitamin B_{12} administered parenterally was as effective as liver extract and is preferable because the pure crystalline substance can be given in smaller volumes of solution and therefore causes less discomfort at the site of injection. Moreover hypersensitivity reactions are not uncommon after injection of liver extract, but single amounts of crystalline vitamin B_{12} as large as 1,000 mcg have often been given intravenously subcutaneously and intramuscularly without a single instance of undesirable effect. In addition, vitamin B_{12} is less expensive than liver extract.

In 11 patients the administration of single oral doses of vitamin B_{12} (1 to 10 mg.) gave results comparable to those obtained with much smaller doses given parenterally An attempt is being made to work out a schedule for oral treatment, but this is still in the experimental stage. One patient has been maintained for 20 months on 2 mg of B_{12} by mouth every four weeks after the initial dose of 5 mg. (1 mg. every four weeks proved insufficient) Another patient has been maintained on 1 mg. every four weeks for 14 months, and 15 others are receiving 1 mg. orally every week, but the observation period is not yet long enough for evaluation of results.

In a group of patients with subacute combined degeneration, neurologic abnormalities of many years' standing were not affected by vitamin B_{12} given parenterally in doses of 150 to 1,000 mcg. at intervals of one to four weeks over a period of five to seven months. Five of 6 patients with less severe neurologic manifestations improved during treatment with 150 mcg. of vitamin B_{12} parenterally at intervals of two to four weeks for 8 to 29 months. Improvement was no greater than in other patients who received only 45 mcg. at six week intervals.

The thiocyanate derivative of vitamin B_{12}, MK-50, was used in one patient with pernicious anemia. Response to a single intramuscular injection of 25 mcg. was prompt, and the patient was maintained in complete remission by injection of 45 mcg. every six weeks for as long as the material was available (10 months) Since then the patient has been maintained on B_{12}.

Johns Hopkins University and Hospital Baltimore, Md.

149 BASTRUP MADSEN, P *Deficiency of antipernicious anemia principle as cause of chronic glossitis without megalocytic anemia*, Nord. med. 48 1444- Oct. 17 1952 (abstr J.A.M.A. 151 523-524 Feb. 7 1953)

"Bastrup-Madsen reports on five patients in whom glossitis was regarded as a manifestation of latent pernicious anemia although megalocytic anemia was absent. The lingual symptoms had lasted from 6 months to 15 years before the start of specific antipernicious anemia treatment. The bone marrow showed maturation disturbances in the myeloid series of the type seen in pernicious anemia. In all cases improvement in the lingual symptoms occurred from 5 to 10 days after specific treatment was begun, with complete disappearance of the symptoms in one of the three cases in which liver treatment was given and in the two cases in which vitamin B_{12} was given. The color index approached 1. In the four cases in which the bone marrow was examined after specific treatment for two weeks the bone marrow picture had returned to normal. As in every other form of pernicious anemia, specific treatment once started must be continued. Interruption of the treatment may lead to the development of irreparable tongue and nerve lesions. "

150 MURPHY W P., and HOWARD, L: *A comparison of the effect of vitamin B_{12} with that of liver extract in the treatment of pernicious anemia during relapse and for maintenance* New England J Med. 247 838-840, Nov 27 1952.

Clinical effects of vitamin B_{12} and liver extract were found to be similar in pernicious anemia patients both during relapse and while on maintenance therapy Of the patients treated during relapse, 8 received intramuscular injections of crystalline vitamin B_{12}, and 20 received liver extract. Reticulocyte levels in the vitamin B_{12} treated group compared favorably with those in the group treated

with liver extract, though comparison of erythrocyte counts showed an almost uniformly greater increase at all initial levels in the patients treated with liver extract. The 8 patients who were treated with vitamin B_{12} during relapse continued to receive it for maintenance therapy All have remained in excellent health with erythrocyte levels within the normal range.

Nine patients who had previously been maintained on liver extract were changed to vitamin B_{12}, and have been observed for periods ranging from 16 months to three years. The average erythrocyte counts were higher for all patients on B_{12} therapy than on liver extract therapy One patient in the maintenance group was returned to liver therapy because of severe central nervous system involvement with locomotor difficulty which did *not appear to be well controlled* by vitamin B_{12}. Severe neural disturbance present in 2 other patients receiving vitamin B_{12} did not progress. The authors suggest that 1 U.S.P anti-pernicious anemia unit of liver extract is equivalent to 1.3 mcg. of vitamin B_{12}.

Peter Bent Brigham Hospital, and Harvard Medical School Boston, Mass.

151. SCHRUMPF A. *Vitamin B_{12} and folic acid in small doses orally in pernicious anemia*, Nord. med. 48: 1621, Nov 1952.

152. THEDERING and RIETHMULLER: *Acido folico y vitamina B_{12} en las anemias megaloblasticas (Folic acid and vitamin B_{12} in megaloblastic anemias)* Folia Clin. Internacional 2 371, 1952 (abstr Orientación méd. 1 805, June 26, 1953)

The combination of folic acid and vitamin B_{12} in pernicious anemia produced results decidedly superior to those achieved with either drug alone. The results are most evident in long term maintenance therapy of pernicious anemia. The authors advise immediate use of this substance in all cases there is no danger in the use of the combination.

153. HECK, F J *Treatment of anemia*, Minnesota Med. 36 40-44, Jan. 1953.

In a review of the types of anemias and their treatment, it is stated that for patients with pernicious anemia, "There is no reason to believe that vitamin B_{12} will not do everything that a good potent liver extract will do and by substituting 15 micrograms of vitamin B_{12} for the 15 units of liver extract, basically the same result will be obtained."

Mayo Clinic Rochester Minn

154 REISNER, E. H., Jr., and WEINER, L. *The treatment of pernicious anemia with massive parenteral doses of vitamin B_{12}*, Blood 8 81-85 Jan. 1953.

Studies were undertaken to determine the effectiveness of single massive injections of vitamin B_{12} in pernicious anemia in relapse and in stabilized cases of combined system disease. Fourteen patients with pernicious anemia in relapse received single intramuscular injections of 1,000 mcg. of vitamin B_{12}, followed by complete hematologic remissions ranging from three to seven months in all but one case. Seven patients in hematologic remission with combined system disease received weekly injections of 1,000 mcg of vitamin B_{12} for periods ranging from four to thirteen weeks. Four patients showed no evidence of objective improvement and one patient resumed liver injections because she felt better on that therapy one patient obtained continued improvement in cord disease, *and another who had had symptoms of combined sclerosis* for four years showed moderate improvement, which was not progressive, *in gait and balance. The amount of vitamin* B_{12} recovered 48 hours after injection from the urine of the patients treated in relapse ranged from 51 to 98 per cent of the injected dose.

The authors draw the following conclusions "These studies indicate that massive single injections of vitamin B_{12} cannot be substituted for more frequent regular injection of smaller doses in the treatment of pernicious anemia, without the danger of relapse and the aggravation of central nervous system disease. Furthermore, there is no evidence to indicate that a greater degree of improvement follows the regular weekly injections of massive doses of vitamin B_{12} to patients with chronic combined system disease than occurs with regular conventional doses. This is probably because amounts of B_{12} above a threshold value of 25 to 50γ by injection, are rapidly and quantitatively excreted in the urine."

Included in the discussion is the report of remarkable improvement in a previously intractable case of sciatica secondary to arthritis with daily doses of 1,000γ of vitamin B_{12} given intramuscularly It is concluded that in such a case the mechanism of action of B_{12} was different from that in combined system disease resulting from pernicious anemia.

New York University Post-Graduate Medical School, and Bellevue Hospital New York, N Y

155 MEACHAM, G. C., and HEINLE, R. W *Maintenance therapy of pernicious anemia with vitamin B_{12}*, J Lab. & Clin. Med. 41 65-77 Jan. 1953.

Authors summary "Patients with pernicious anemia were given maintenance therapy with crystalline vitamin B_{12}, and a concentrate containing vitamin B_{12} and vitamin B_{12b} for periods up to sixteen months. In the dosages used, these substances were as effective as purified liver extract.

"An average daily dose of 1 μg. given intramuscularly at intervals of three or four weeks appeared to be sufficient to maintain normal erythrocyte levels and to prevent the development or progression of neurologic or lingual lesions.

"In previous experiments, the concentrate was less effective than the crystalline preparation, but with the concentrates available now there is probably no significant difference.

"Mild macrocytosis persisted in many of the patients and was not altered by the intramuscular administration of purified liver extract nor by the addition of daily oral doses of folic acid. Comparison *of the treated patients* with a series of normals' from the literature indicates that the relationship of the erythrocyte level to
value in the two series is different, even when
tients have high normal' blood valu

"This suggests the possibility that neither vitamin B_{12} purified liver extract, nor folic acid given singly or in combination completely corrects the erythrocyte abnormality in pernicious anemia and suggests that some other substance(s) may be necessary in addition.

"From the practical standpoint, however treatment with sufficient amounts of vitamin B_{12}, B_{12} concentrates, or purified liver extract maintains normal erythrocyte levels and prevents development or progression of neurologic and lingual lesions."

Western Reserve University School of Medicine, and University Hospitals Cleveland, Ohio

156. FIELD R.: *Cases from the Medical Grand Rounds (Cases 235 and 236)* Am. Pract. 4 143-146, Feb. 1953.

Two cases of pernicious anemia are presented and discussed in detail. In one patient the reticulocyte count rose about 10 per cent on 100 mcg. of vitamin B_{12} intramuscularly daily However treatment was changed to liver extract since it was thought that the patient's response to B_{12} was slow Lack of striking response to either agent was ascribed to an active bladder infection. The author feels that one U.S.P unit of liver is equivalent to 3 to 5 mcg. of vitamin B_{12}. Thus a maintenance dose is 30 U.S.P units of liver extract per four weeks, or at least 100 mcg. of vitamin B_{12}.

Massachusetts General Hospital Boston, Mass.

157 MONTO R. W., REBUCK, J W., and BRENNAN M. J *Crystalline B_{12} inhalation therapy in pernicious anemia*, Am. J M. Sc. 225 113-119 Feb. 1953.

Crystalline vitamin B_{12} given by inhalation to 3 patients with pernicious anemia caused adequate clinical and hematologic responses. Two patients received inhalations of vitamin B_{12} in saline administered by pressure tank and a vaponephrin nebulizer The third received vitamin B_{12} and lactose powder by means of a dust inhaler Two of the patients had severe and the other mild pernicious anemia. One had symptoms indicating an early stage of subacute combined degeneration of the cord.

The dosage schedule was similar to that used for parenteral therapy The lungs were not adversely affected. Detectable amounts of B_{12} activity were found in the urine after therapy

In an addendum it is stated that 3 patients with pernicious anemia in relapse have been adequately treated by inhalation of crystalline vitamin B_{12}.

The material given by aerosol contained 15 mcg. of vitamin B_{12} per cc. The dust mixture contained 1,000 mcg. of vitamin B_{12} in 0.1 cc. of lactose powder

Henry Ford Hospital Detroit, Mich.

158. ÖSTLING, G., NYBERG W., and GORDIN R.: *Antianemic activity of alkali-treated crude liver extract*, Acta med. Scandinav 154 40-43, March 23, 1953.

In a clinically effective "crude" liver extract which contained 3.5 mcg. of vitamin B_{12} per cc., folic acid, folinic acid and desoxyribosides in amounts too small to be clinically effective, and two microbiologically effective alkali stable factors, the vitamin B_{12} was destroyed by boiling with alkali. After this treatment the liver extract was found to have the same therapeutic effect in pernicious anemia as extract which had not been treated with alkali. Three cases are reported, 2 of cryptogenetic pernicious anemia and 1 of pernicious anemia which may have been the result of tapeworm infestation.

Maria Hospital Helsingfors, Finland

159 ANNOTATION *Maintenance treatment in pernicious anaemia*, Lancet 1 782-783, April 11, 1953.

After a review of seven recent references to the successful use of vitamin B_{12} in pernicious anemia, the annotation is concluded with this paragraph:

"Vitamin B_{12} provides a safe and sure means of maintaining in good health patients with classical pernicious anaemia. If it fails to give good maintenance, then complications must be sought or the diagnosis reconsidered. With vitamin B_{12} and the standard liver extracts freely available, there should in our view be no need to use so-called complete haematinics. Folic acid is commonly one constituent of these and a patient with pernicious anaemia who is given folic acid is exposed to the risk of affection of the central nervous system—a risk that is surely unjustifiable when safe treatments are to hand."

160. BOUCHER, R., and PROULX, A.: *L anémie pernicieuse et son traitement présentation d'un cas classique (Pernicious anemia and its treatment presentation of a classic case)* Union méd. du Canada 82 380-385, April 1953.

Good response to vitamin B_{12} in a case of pernicious anemia is reported. The dosage was 15 mcg. a day parenterally Alternating the B_{12} with 15 units of calves liver did not hasten response. After the seventh week the dosage was reduced to 15 mcg. weekly Two weeks later the patient insisted on leaving the hospital. She was advised to take 15 mcg. of vitamin B_{12} every two weeks.

Hospital Saint-Luc Montreal, Canada

161. MEYER, L. M., SAWITSKY A., RITZ, N. D., and BRAHIN C.: *Treatment of pernicious anemia with crystalline vitamin B_{12b}*, Blood 8 358-369 April 1953.

Crystalline vitamin B_{12b}, was given intramuscularly to 9 patients with pernicious anemia in relapse. A daily dose of 1.0 mcg. for 35 to 210 days gave an unsatisfactory clinical and hematologic response in 6 patients. In 3 patients the daily dose of vitamin B_{12b} was 2 mcg. for 90 to 190 days; satisfactory clinical and hematologic remissions were obtained. "Crystalline vitamin B_{12b} is probably less potent as an anti-anemia agent than crystalline vitamin B_{12}."

Goldwater Memorial Hospital Welfare Island, N Y Veterans Administration Hospital Bronx, N Y

162. SAWITSKY A., MEYER, L. M., McINERNY R., and DIEFENBACH, W. C. L.: *Oral treatment of pernicious anemia with citrovorum factor (Leucovorin)* Acta med. Scandinav 145 332-337 May 27 1953.

Five patients with pernicious anemia in relapse were treated with citrovorum factor in daily oral doses of 0 15 to 15.0 mg. One patient failed to respond and another responded but relapsed after two months; both these patients responded to intramuscular injections of vitamin B_{12}. Two patients showed suboptimal reticulocyte peaks but were not followed for long enough periods to determine the maximal hematologic effect. One patient attained normal blood levels and was in good condition six months after the institution of therapy Symptomatic improvement accompanied hematologic improvement in each case.

Queens General Hospital
Jamaica, N Y
Veterans Administration Hospital
Bronx, N Y

163. MEYER, L. M., McINERNY R., and RITZ, N D: *Intravenous treatment of pernicious anemia with vitamin B_{12}*, J Clin. Nutrition 1 299-301 May June 1953.

Authors conclusions: "Daily intravenous administration of 0.5 or 1.0 g. of vitamin B_{12} to 5 patients with pernicious anemia in relapse induced a satisfactory clinical remission.

"Erythrocyte regeneration was satisfactory in 1 case (receiving 0.5 g. per day) and greater than anticipated in 4 cases (receiving 1.0 g. per day)

"Erythremic levels were attained in 2 patients treated for 41 and 87 days with 1.0 g. of vitamin B_{12} daily"

Veterans Administration Hospital
Bronx, N Y
Swedish Hospital
Brooklyn, N Y

ANEMIAS IN

PREGNANCY PUERPERIUM

164 DAY L. A., HALL, B. E., and PEASE, G L. *Macrocytic anemia of pregnancy refractory to vitamin B_{12} therapy response to treatment with folic acid report of case*, Proc. Staff Meet., Mayo Clin. 24 149-157 March 30, 1949

A patient with macrocytic anemia of pregnancy was treated with vitamin B_{12} after treatment with liver extract had been unsuccessful. Vitamin B_{12} was started on the 24th day of illness and was given parenterally in a total amount of 27.5 mcg. over eight days. No hemopoietic response occurred. The patient became nauseated and vomited after each injection of vitamin B_{12}. She vomited all afternoon following the last injection and passed 13 watery stools.

On the 32nd day of illness, folic acid therapy was started. The initial dose was 30 mg. parenterally and each day thereafter (except for one day a week) 15 mg. of folic acid was given until delivery occurred one month later Both the hemopoietic and clinical responses to folic acid were excellent.

Mayo Clinic
Rochester Minn.

165 FURMAN R. H., DANIELS W B., Jr., HEFNER, L. L., JONES E., and DARBY W J: *Pernicious anemia of pregnancy failure of vitamin B_{12} therapy successful treatment with folic acid report of a case* Am. Practitioner & Digest of Treatment 1 146-147 Feb. 1950.

A case of so-called pernicious anemia of pregnancy is reported which failed to respond to vitamin B_{12} treatment (10 mcg. a day intramuscularly for a week) Folic acid treatment was then given in intramuscular doses of 15 mg. daily for 20 days the patient responded well. Three similar cases have been reported in the literature.

The resemblance of this type of anemia to the tropical macrocytic anemia described by Wills has long been evident, and evidence is increasing for the identity of vitamin M, "Wills factor" and folic acid. The existence of a group of anemias with megaloblastic bone marrow which responded to folic acid but not to parenteral liver therapy or vitamin B_{12} should be recognized.

Vanderbilt University School of Medicine
Nashville, Tenn.

166. HOFMEISTER, F J., STOUFFER, J G., and STEHLIN J S. *Pernicious anemia of pregnancy* Wisconsin Med. J 49 129- Feb. 1950.

167 GINSBERG V., WATSON, J., and LICHTMAN H.: *Megaloblastic anemia of pregnancy response to pteroylglutamic acid after failure of response to liver extract and vitamin B_{12}*, J Lab. & Clin. Med. 36 238-241, Aug. 1950.

The authors describe a case of megaloblastic anemia of pregnancy which failed to respond to refined liver extract or to vitamin B_{12}, in a dosage of 1 mcg. a day given intramuscularly for 13 days. No adverse reactions to vitamin B_{12}, were observed, and a severe glossitis cleared up after one week of therapy The patient subsequently responded maximally to therapy with folic acid, which was given intramuscularly in a dosage of 15 mg. three times a week.

Kings County Hospital
Brooklyn, N Y

168. HOLLY R. G *Megaloblastic anemia in pregnancy Remission following combined therapy with ascorbic acid and vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 78 238-241 Oct. 1951

Megaloblastic anemia of pregnancy is refractory to vitamin B_{12}. It may be related to ascorbic acid deficiency —all 5 cases reported here occurred in April and May Determinations of plasma ascorbic acid levels were made in 3 of these patients the values were decreased or zero. Three of the patients failed to respond to vitamin B_{12} or to ascorbic acid alone, but a combination of the two agents gave a complete hematologic remission. The fourth patient responded to combined folic acid and transfusion therapy and the fifth had a spontaneous remission following delivery

University of Minnesota Medical School
Minneapolis, Minn.

169 CHAUDHURI, S.: *Vitamin B_{12} in megaloblastic anaemia of pregnancy and tropical nutritional macrocytic anaemia*, Brit. M. J 2 825-828, Oct. 6, 1951

Author's summary "Sixteen cases of megaloblastic anaemia of pregnancy and the puerperium were treated successfully with parenteral vitamin B_{12}. Out of five cases of tropical megaloblastic anaemia treated with vitamin B_{12}, four responded to vitamin B_{12} and one responded better to subsequent folic acid therapy

"Four micrograms of vitamin B_{12} a day was the estimated requirement in mild cases of macrocytic anaemia of pregnancy and 10 µg. in severe cases."

Lady Hardinge Medical College
New Delhi, India

170. WOODRUFF A. W *Anaemia of pregnancy among Africans in Nigeria*, Brit. M. J 2 1415-1423, Dec. 15, 1951

In an article concerning anemia of pregnancy among Africans it is reported that an injection of 80 mcg. of vitamin B_{12} was given without response to a case of normocytic anemia. Folic acid failed also. Of 2 cases of macrocytic anemia given 120 mcg. and 100 mcg., respectively one responded slightly and the other not at all. A patient with macrocytic anemia who was given 100 mcg. of vitamin B_{12} six days after delivery improved, but apparently no faster than those patients having natural remissions.

University College
Ibadan, Nigeria

171. FOY H., KONDI, A., and HARGREAVES, A.: *Response of the megaloblastic anaemias of pregnancy to animal protein factor preliminary report*, Brit. M. J 1 852-853, April 19 1952.

In a report of one case the authors suggest that "the response may have been due to (a) changes in the bacterial flora of the gut brought about by the antibiotic present in the A.P.F ; (b) an unknown haemopoietic factor present in the A.P.F (c) the small amount of vitamin B_{12} present in the A.P.F ; or (d) sparing action of the aureomycin [in A.P.F] on the vitamin B_{12}."

Wellcome Trust Research Laboratories, and
Colonial Medical Service
Kenya, Africa

172. O'GRADY J W., RIVA, H. L., and RITZENTHALER, J G. *Hemolytic anemia in pregnancy* Am. J Obst. & Gynec. 65: 1338-1344 June 1953.

Two cases of hemolytic anemia associated with pregnancy are reported. One patient was treated conservatively (transfusion, vitamin B_{12} and folic acid) The second patient was treated by splenectomy The first patient had a premature living child, the second a normal baby

Walter Reed Army Hospital
Washington, D. C.

173. PATEL, J C., and KOCHER, B. R.: *Vitamin B_{12} in macrocytic anaemia of pregnancy and the puerperium*, Brit. M. J 1 924-927 April 22, 1950.

Five cases of macrocytic anemia of pregnancy and the puerperium were treated with vitamin B_{12} by intramuscular injection. In all of the cases a 40-mg. dose produced moderately good to striking response which reached a maximum on the tenth day A 20-mg. dose, tried in 1 of the patients, produced partial improvement, both clinical and hematologic. The authors believe a further increase in the dosage would probably bring the blood picture to normal.

King Edward Memorial Hospital
Bombay India

174 UNGLEY C. C., and THOMPSON R. B. *Vitamin B_{12} and folic acid in megaloblastic anaemias of pregnancy and the puerperium*, Brit. M. J 1 919-924, April 22, 1950.

In 6 patients with megaloblastic anemia associated with pregnancy or the puerperium, the injection of vitamin B_{12}, in doses of 65 to 80 mcg., had no effect. All the patients responded to the subsequent oral administration of folic acid, in daily doses of 2.5 or 20 mg. In one serious case a 80-mg. dose of folic acid was given intravenously

Royal Victoria Infirmary and
King's College
Newcastle-upon-Tyne, England

175 THOMPSON R. B., and UNGLEY C. C. *Megaloblastic anemia of pregnancy and the puerperium*, Quart. J Med. 20 187 204, 1951 (abstr Blood 6 1300-1301 Dec. 1951)

"This report includes observations on 45 patients during the past seventeen years, 26 of whom were followed for periods of from one to fourteen years. The clinical features were essentially those previously described by these and other authors.

"The response to therapy reported here further emphasizes the differences between megaloblastic anemia of pregnancy and Addisonian pernicious anemia. This observation is contrary to the theory that megaloblastic anemia of pregnancy is due to a deficiency of the anti-pernicious anemia principle, brought about either by lack of extrinsic factor from the diet or by a temporary absence of intrinsic factor from the stomach. All 8 patients who received folic acid responded. Responses were also obtained to raw liver pulp and to yeast extract.

"The dietary histories were investigated in 27 cases in only 11 of these could the anemia be explained on a deficiency basis.

"The survival rate of transfused normal erythrocytes was followed in three cases and in each there was an increased rate of elimination."

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

176. EDITORIAL *Folic acid deficiency in macrocytic anemia of pregnancy and infancy* J.A.M.A. 148 1422, April 19 1952.

The role of folic acid in the prevention and treatment of macrocytic anemia is discussed in an editorial with eight references It has been demonstrated that there is a physiologic relationship between the failure of hemopoiesis in the mother and failure of hemopoiesis in the nursing infant due to a deficiency of folic acid. Lack of folic acid explains why certain patients did not respond to the administration of the purified anti-pernicious anemia liver extract. Most potent antianemia liver extracts

are rich in vitamin B_{12} but are almost devoid of folic acid. Liver extract, vitamin B_{12}, and folic acid each fail in the treatment of some types of macrocytic anemia.

"Nothing takes the place of diagnostic acumen and appreciation of the activity and specificity of the various vitamins. The fact that folic acid does not relieve acute neural degeneration is not a general indictment against it, for its value in some types of macrocytic anemia is beyond dispute. Likewise the fact that vitamin B_{12} does not always relieve the macrocytic anemia of pregnancy and the megaloblastic anemia of infancy is no indictment against it. Vitamin B_{12}, or substances that act similarly are essential for the integrity of the nervous system. Folic acid is essential in some types of macrocytic anemia hence, both vitamin B_{12} and folic acid are essential for life."

NEWBORN

177 ANDERSON D E. *Chronic aregenerative anemia of the newborn*, M. J Australia 1 573-576, April 26, 1952 (abstr Am. J Digest. Dis. 20 88, March 1953)

Chronic aregenerative anemia of the newborn (chronic hypoplastic anemia, congenital hypoplastic anemia) *is not benefited by folic acid, liver extract and* vitamin B_{12}. Repeated blood transfusions permit the patient to live for many years but the ultimate prognosis is not yet known.

INFANCY

178. McPHERSON A. Z., JONSSON U., and RUNDLES, R. W *Vitamin B ₁₂ therapy in megaloblastic anemia of infancy* J Pediat. 34 529-536, May 1949

Two infants with megaloblastic anemia have been successfully treated with vitamin B_{12}. One was a Negro aged 11 months. Two days after a single intramuscular injection of 0.002 mg. of vitamin B_{12}, her reticulocyte percentage rose and reached a peak of 69.1 per cent on the seventh day A bone marrow examination made at this time showed a suggestion of megaloblastic development, but on the thirteenth day after injection another examination showed normoblastic cellular development with active cell proliferation. The regeneration of the blood was rapid. The child was discharged two weeks after the injection. Follow-up examinations 3 and 10 weeks later showed weight gain and normal blood values.

The other child, aged 7 months, was given 0.002 mg. of vitamin B_{12} intramuscularly and a week later 0.005 mg. On the twelfth day the dosage was increased to 0.002 mg. daily and continued for 18 days. Serum albumin was given intravenously for four days. By the nineteenth day improvement was evident. The patient was discharged 32 days after the beginning of treatment and appeared perfectly well at a follow-up examination six weeks later

179 WOODRUFF C. W., RIPY H. W., PETERSON J C., and DARBY W J *Variable response to vitamin B_{12} of megaloblastic anemia of infancy* Pediatrics 4 723-729 Dec. 1949

Two infants with megaloblastic anemia showed a good hematologic response to vitamin B_{12} (0.025 mg. given subcutaneously in one case and intramuscularly in the other) A third child with this disease did not respond to B_{12} but subsequently responded to folic acid. It is suggested that megaloblastic anemia of *infancy may be a* syndrome rather than a single entity

An addendum states that 2 additional patients with megaloblastic anemia have been treated with vitamin B_{12}. One responded to this vitamin the other did not, but experienced a remission when folic acid was given.

Vanderbilt University School of Medicine
Nashville Tenn.

180. LUHBY A. L., and WHEELER, W E. *Megaloblastic anemia of infancy II—Failure of response to vitamin B_{12}, and the metabolic role of folic acid and vitamin C*, Health Center J 3 1 20, Dec. 1949

Four infants with megaloblastic anemia (which occurs in early infancy is usually corrected with a single course of *either liver extracts or folic* acid, *and does* not recur or produce changes in the central nervous system) were treated with vitamin B_{12}. The vitamin was given either parenterally or orally in the form of a natural source (ground beef) Three patients were completely refractory to large doses of vitamin B_{12} (0.025 to 0.1 mcg.) intramuscularly Their subsequent response to folic acid, refined liver extract, vitamin C, and certain food substances suggest that both pteroylglutamic acid (PGA) and vitamin B_{12} are necessary for the nucleoprotein metabolism of blood formation and that even large doses *of one of these substances are ineffective if the other* is not physiologically available. Relatively large doses of one may enhance the effect of small amounts of the other but if the deficiency of one is great enough the other cannot produce an effective hematopoietic response.

The role of vitamin C in megaloblastic anemia of *infancy is probably not a direct hematopoietic* action but a sparing effect on PGA.

The primary cause of megaloblastic anemia of infancy appears to be, in most cases, a physiologic deficiency *of* PGA. *Factors involved in its development* are inadequacies in the diet, infection, and a low vitamin C intake, all of which reduce or keep at a low level the folic acid reserves which are available for blood formation. The authors feel, therefore, that folic acid is the therapeutic agent of choice in the treatment of this disease, and that vitamin B_{12} should not be the sole therapeutic agent. Growth disturbance in infants with this disease is presumably due to an associated aberration of their protein metabolism.

Ohio State University College of Medicine, and
Children's Hospital
Columbus, Ohio

181 STURGEON P., and CARPENTER, G *Megaloblastic anemia of infancy Response to vitamin B_{12}*, Blood 5 458-467 May 1950.

It was found that *3 cases of* megaloblastic anemia of infancy showed a specific and complete response to a single intramuscular dose of 25 mcg. or less of vitamin B_{12}. Two other cases showed equivocal responses to similar treatment. It is concluded that vitamin B_{12} is thera

peutically effective in some cases of megaloblastic anemia of infancy It is suggested that vitamin B_{12} therapy may be made more effective by concomitant therapy with ascorbic acid.

Children's Hospital, and
University of Southern California
Los Angeles, Calif.

182. MAY C. D., NELSON E. N., LOWE, C. V., and SALMON R. J *Pathogenesis of megaloblastic anemia in infancy An interrelationship between pteroylglutamic acid and ascorbic acid,* Am. J Dis. Child. 80 191 206, Aug. 1950.

University of Minnesota Medical School
Minneapolis, Minn.

183. MAY C. D., NELSON E. N., SALMON R. J., LOWE, C. V., LIENKE, R. J., and SUNDBERG R. D *Experimental production of megaloblastic anemia in relation to megaloblastic anemia in infants,* Bull. Univ Minnesota Hospitals 21 208, 1950 (cited in Blood 5 714 Aug. 1950)

184 WOODRUFF C. W., and PETERSON J C. *The treatment of megaloblastic anemia in infancy* Post grad. Med. 10 189-191, Sept. 1951.

Infants that develop megaloblastic anemia have frequently been fed almost exclusively on milk or formulas largely derived from milk. Infections and inadequate intake of ascorbic acid are often precipitating factors. Folic acid is effective given parenterally in large doses (5 to 15 mg. daily) or orally The authors have seen adequate response to as little as 0.2 mg. a day orally Vitamin B_{12} is effective in many cases but is sometimes completely ineffective in others. For uncomplicated megaloblastic anemia of infancy the authors recommend 5 mg. of folic acid daily by mouth. At least 100 mg. of ascorbic acid should be given daily or its equivalent as orange juice.

Vanderbilt University School of Medicine
Nashville, Tenn.

185. RUD E.: *Case of macrocytic goat's milk anemia successfully treated with vitamin B_{12}* Ugesk. f. laeger 113 1301 Sept. 27 1951

186. BETKE, K., and GANTERT L. *Etiology and therapy of goat's milk anemia,* Deutsche med. Wchnschr 76 1341 Oct. 26, 1951 (abstr J.A.M.A. 148 403, Feb. 2, 1952)

The term "goat's milk anemia" should be applied only to anemia in which a megalocytic blood picture and megaloblastic transformation of the bone marrow develop during the feeding of goat's milk. This anemia resembles pernicious anemia. Case histories are given for 2 infants having goat's milk anemia. Treatment with folic acid and vitamin B_{12} effected recovery The course of the reticulocytic crisis indicated that the anemia resulted from deficiencies of both vitamin B_{12} and folic acid.

187 RICKARDS, A. G.: *Megaloblastic anaemia of infancy* Brit. M. J 1 1226-1227 June 7 1952.

An 8 month old baby girl with megaloblastic anemia of infancy responded to folic acid and vitamin B_{12}. Five months later she relapsed, and this time she was treated with folic acid. She is now receiving a maintenance dosage of 10 mg. of folic acid daily

Other cases of megaloblastic anemia of infancy and juvenile pernicious anemia appearing in the literature are reviewed briefly and the etiology of megaloblastic anemia of infancy is discussed.

Royal Lancaster Infirmary
England

CHILDHOOD

188. DAVIS R. W., CHRISTIAN R. M., ERVIN D. M., and YOUNG L. E. *Pernicious anemia in childhood report of case in six year old girl responding to refined liver extract, folic acid and vitamin B_{12} in successive relapses* Blood 4 1361 1366, Dec. 1949

This case presentation of megaloblastic anemia without specific neurologic complications in a 6 year old girl provides an example of childhood pernicious anemia It is regarded as such despite the fact that a small amount of free hydrochloric acid was present in the gastric juice after injection of histamine. Following administration of refined liver extract, folic acid and vitamin B_{12} in successive relapses, prompt hematologic responses, which were similar in each case, were obtained. The patient was given 12.5 mcg. of vitamin B_{12} intramuscularly daily for six days.

University of Rochester School of Medicine and Dentistry
Rochester N Y

189 ROSENZWEIG L., and BRUTON O C. *Pernicious anemia in an eight year old girl additional observations in a case previously reported as "Nutritional anemia in an infant responding to purified liver extract,"* Pediatrics 6 269-276, Aug. 1950.

Additional studies of a case originally reported by Fouts and Garber (*Am. J Dis. Child.* 75: 143, 1948) as nutritional anemia in an infant show it to be a case of pernicious anemia in childhood. Up to the age of 8 years the child was given liver extract. This therapy was then discontinued to determine whether the child still required it. After two months, anemia developed. Folic acid treatment, 15 mg. a day by mouth, was given for 24 days, with good results. After about four months the patient was readmitted to the hospital and given vitamin B_{12}, 10 mcg. at weekly intervals. Response was prompt, and this treatment is being continued since the patient prefers it to liver extract, which has sometimes caused mild reactions and gives more pain at the site of injection than does vitamin B_{12}.

Walter Reed General Hospital
Washington, D. C.

190. REISNER, E. H., JR., WOLFF J A., McKAY R. J., JR., and DOYLE, E. F: *Juvenile pernicious anemia,* Pediatrics 8 88-106, July 1951

Macrocytic anemia associated with megaloblastic bone marrow resulting from failure of absorption of vitamin B_{12} due to lack of the intrinsic factor of Castle present in normal gastric juice, is known as addisonian

pernicious anemia. This disease, most common in the sixth decade and ordinarily associated with histamine-refractory achlorhydria, has seldom been reported in children; in only 7 cases from the literature can this diagnosis be accepted if the absence of hydrochloric acid after the administration of histamine is specified as a criterion. The authors, however, believe that intrinsic factor deficiency may occur without achlorhydria.

Case histories are presented of 4 children who had what is believed to be true pernicious anemia although achlorhydria was not a factor in 3 of them. All 4 patients responded to liver extract and to vitamin B_{12} given parenterally In 3 to whom vitamin B_{12} was given by mouth no response was obtained. Two of them, however subsequently responded to oral B_{12} with normal gastric juice. The other 2 patients developed histamine refractory achlorhydria while in hematologic remission, which is interpreted as proof that the anemia was due to deficient intrinsic factor in the gastric juice. Three of these patients showed evidence of disease of the spinal cord In 2 of them symptoms were severe. The symptoms were compatible with a diagnosis of dorsolateral sclerosis and improved with anti-pernicious anemia therapy In 1 of these patients, folic acid treatment aggravated the neurologic lesions.

New York University College of Medicine,
Bellevue Hospital,
Columbia University College of Physicians and Surgeons, and
Babies Hospital
New York, N Y

191 PIERCE, M. L.: *Management of anemia in childhood*, Postgrad. Med. 11 68-73, Jan. 1952.

The various anemias of childhood are discussed. Among the treatments suggested are the administration of folic acid in "goat's milk" nutritional anemia, folic acid in megaloblastic anemia in infancy with ascorbic acid if there is a deficiency in this vitamin, ACTH in acquired hemolytic anemia, with repeated transfusions and even splenectomy in chronic cases, and ACTH in some secondary types of aplastic and hypoplastic anemias, particularly those due to noxious agents. In the summary it is stated that vitamin B_{12} is one of the agents indicated in anemias in children; specific mention of its use is in the section on megaloblastic anemia, in which this vitamin is said to be less effective than folic acid.

University of Chicago, and
Bobs Roberts Memorial Hospital
Chicago, Ill.

OLD AGE

192. BASYLEWYCZ, I. V: *Pernicious anemia in old age*, Rhode Island M. J 34: 641-644, 664, Dec. 1951.

The author believes that since old people, as a rule, do not respond to liver therapy as promptly as younger patients, the newer treatment of pernicious anemia, with use of vitamin B_{12}, has a great future in the aged, as well as in young people. Concomitant administration of B complex vitamins is especially valuable in elderly patients. Causes of vitamin B_{12} deficiency in the aged include inadequate diet, insufficient assimilation of available vitamin B_{12} resulting from difficulties in chewing, gastric achylia and pancreatic disorders, possible inability of the liver to store vitamin B_{12}, and possible impaired utilization of vitamin B_{12} by aging tissues.

Rhode Island State Hospital for Mental Diseases
Howard, R.I.

OTHER ANEMIAS

193 SEBRELL, W H: *Anemias caused primarily by malnutrition*, Federation Proc. 8 568-578, June 1949

The literature is reviewed with special reference to the microcytic or normocytic anemias associated with riboflavin, pyridoxine, pantothenic acid, niacin, amino acid, and vitamin C deficiencies, and with reference to the macrocytic anemias and the role of pteroylglutamates and vitamin B_{12}.

194 KERPPOLA, W *Observations on the phosphatase content of blood and bone marrow cells in normal and pathologic hemopoiesis*, Blood 6 454-465, May 1951.

In this article reference is made to a case of "achrestic" anemia in which the patient is described as having responded weakly to treatment with vitamin B_{12}.

University of Helsinki
Helsinki, Finland

195. DASHER, W A., and BOGEN E.: *Antianemic factors in the treatment of iron deficiency anemias*, Ann. West. Med. & Surg 6 85-87 Feb. 1952.

The addition of the so-called antianemic factors (folic acid, vitamin B_{12}, powdered stomach and liver fraction) to iron in the treatment of patients with iron deficiency anemias did not result in greater improvement In fact the patients on iron alone did better than the others. Indiscriminate use of antianemic factors is wasteful and contraindicated in treatment of iron deficiency anemias.

Burbank, Calif. (W.A.D.)
Olive View, Calif. (E.B.)

196. SILVER, H. K. *Mediterranean anemia*, California Med. 76 162 164, March 1952.

History, incidence and distinguishing features of the disease are discussed. In its severe form, early and periodic transfusions of whole blood offer the only effective treatment of this anemia. Numerous other types of treatment, including splenectomy B_{12}, liver iron, and other hematinics have not been of value, in the author's experience. He states that in the mild form of this anemia, specific treatment is not necessary

University of California School of Medicine
San Francisco, Calif

197 HUNTER, O B., JR.: *L. citrovorum factor in refractory anemia*, Ann. West. Med. & Surg. 6 275-278, May 1952.

Six cases of anemia refractory to the usual therapeutic measures are reported. Citrovorum factor produced a slight increase in the reticulocyte response over that resulting from the use of folic acid or vitamin B the effect was not maintained after therapy w tinued, and a second course was not effectiv

case. In one patient, a blood count considerably better than previously noted was maintained for six months by citrovorum factor There is no indication, however that citrovorum factor will maintain the red cell count on an adequate level.

Doctors Hospital Research Foundation,
Washington, D. C.

198. KJERULF JENSEN K., and SCHWARTZ, M. *Gout, leukaemia, and polycythaemia*, Lancet 1 599 March 21, 1953 (in Letters to the Editor)

A patient was treated with 80 mcg. of vitamin B_{12} and 2 Gm. of hog s stomach extract daily for anemia accompanying gout. After two and one-half months polycythemia developed, its peak occurring two weeks after this treatment was discontinued. The blood count showed red cells 7,600,000 per cu. mm., Hb 121 per cent, leukocytes 26,000 per cu. mm., thrombocytes 316,000 per cu. mm. Sternal marrow biopsy showed intense hyperplasia with a pronounced shift to the left and no megaloblasts suggesting polycythemia. Moderate splenomegaly had developed. No treatment was given for three months, then vitamin B_{12} and hog s stomach extract was given for one month. The bone marrow showed the same picture as before, but the polycythemia did not recur

Rigshospitalet
Copenhagen, Denmark

ANEMIAS IN GENERAL

199. EDITORIAL *Vitamin B_{12} in anemia*, Am. Prof. Pharm. 14 427 May 1948.

200. SPIES, T D., STONE, R. E., and ARAMBURU T: *Observations on the antianemic properties of vitamin B_{12}*, South. M. J 41: 522-523, June 1948.

Two patients with pernicious anemia, 2 with nutritional macrocytic anemia, and 1 with nontropical sprue were given crystalline vitamin B_{12}. Each case had been under observation for years. Four of the patients received a single injection of 6 mcg. 1 patient with pernicious anemia was given 15 mcg. in one dose. Within three to five days all the patients showed striking clinical improvement, which included an increase in their feeling of well being, mental alertness, strength and vigor and complete relief of soreness and burning of the tongue and mouth. There was a rise in reticulocytes in each case, reaching a peak from the fifth to the ninth day This was followed by an increase in red blood cells and hemoglobin and, with the exception of 1 patient with nutritional macrocytic anemia, in white blood cells also. The response in the 2 cases of nutritional macrocytic anemia and in the 1 case of nontropical sprue shows that vitamin B_{12} has positive hemopoietic activity in these diseases as well as in pernicious anemia.

Northwestern University Studies in Nutrition
Hillman Hospital
Birmingham, Ala.

201. SPIES, T D., STONE, R. E., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and ARAMBURU T: *Thymine, folic acid, and vitamin B_{12} in nutritional macrocytic anaemia, tropical sprue and pernicious anaemia*, Lancet 2 519-522, Oct. 2, 1948.

The hematologic responses of the same individual to thymine, folic acid, and vitamin B_{12} were compared in 3 patients with macrocytic anemia in relapse. One patient had nutritional macrocytic anemia, one tropical sprue, and one pernicious anemia. Each patient was admitted to the hospital three times for treatment of anemia in about the same degree of relapse. During the test periods the patients were given a diet free from meat and meat products. Thymine and folic acid were taken orally over periods of 10 or 14 days vitamin B_{12} was administered in a single intramuscular injection. Satisfactory hematologic response and definite clinical improvement were obtained with each of the three substances in each case—in the patient with nutritional macrocytic anemia with 6 Gm. of thymine or 10 mg. of folic acid daily or with 6 mcg. of vitamin B_{12}; in the patient with tropical sprue with 10 Gm. of thymine or 10 mg. of folic acid daily or with 23 mcg. of vitamin B_{12} and in the patient with pernicious anemia with 6 Gm. of thymine or 10 mg. of folic acid daily or with 15 mcg. of vitamin B_{12}. Approximately equal reticulocyte peaks were obtained with folic acid and vitamin B_{12} within similar intervals (about the seventh day in 2 patients and the eleventh day in the third) Except in the patient with pernicious anemia, the reticulocyte response was less pronounced under thymine. With all three substances there was progressive increase in the red blood cell count and hemoglobin level throughout the period of study

The patient with nutritional macrocytic anemia had a more severe glossitis at the time of his third relapse than on the two previous occasions. This disappeared in five days following treatment with vitamin B_{12} and the patient gained strength quickly and steadily The patient with pernicious anemia showed only slight clinical improvement while on thymine and his stomatitis and glossitis remained unchanged. Folic acid had a more favorable effect on these symptoms but they did not disappear entirely Glossitis, stomatitis, and weakness were severe in this patient at the time of his third relapse. Following treatment with vitamin B_{12}, the hyperemia of the tongue and oral mucosa began to fade after 48 hours, and the mucosa appeared almost normal five days after the injection; the papillae had regenerated by the thirteenth day In this case the blood response to vitamin B_{12} was paralleled by remarkable improvement in strength and vigor

From these and their other studies, the authors conclude that, while thymine, folic acid, and vitamin B_{12} are each effective in the above three syndromes, to produce comparable responses the required dose of thymine is several thousand times that of folic acid, and the required dose of folic acid several thousand times the effective dose of vitamin B_{12}. This suggests that vitamin B_{12} is by far the most potent antianemic substance known to date.

Northwestern University Medical School
Chicago, Ill.
Hillman Hospital
Birmingham, Ala.
University of Havana
Havana, Cuba

202. BETHELL, F H., MEYERS M. C., and NELIGH, R. B. *Vitamin B_{12} in pernicious anemia and puerperal macrocytic anemia*, J Lab. & Clin. Med. 33: 1477 1478, Nov 1948 (in Soc. Proc.) (*also in* Proc. Central Soc. Clin. Research 21 27 28, 1948)

Four patients with pernicious anemia in relapse were treated with vitamin B_{12} in daily intramuscular injections of 1 microgram until there was no further reticulocyte response, after which injections were given at varying intervals up to 25 days, the dosage being adjusted to provide the equivalent of 1 mcg. a day Treatment was continued for 10 to 20 weeks. Three of the patients had a greater hematologic response than would have been expected from daily injections of 1 U.S.P unit of liver extract. The fourth patient, who was given 1 mg. of the folic acid antagonist, aminopterin, daily for 2 days before and for the first 14 days during treatment with vitamin B_{12}, had a delayed and suboptimal reticulocyte response, but after aminopterin was discontinued the red cell count rose rapidly All 4 patients had normal hematologic values after six to eight weeks. Paresthesias were relieved and ataxia decreased in 3 patients with nervous system involvement, and glossitis, which was troublesome in 3 patients, disappeared promptly under vitamin B_{12} treatment. One case of macrocytic anemia following pregnancy failed to respond to daily doses of 1 mcg. vitamin B_{12} for 10 days but later responded to 10 mg. of folic acid daily by mouth. Assay of the feces of 4 patients with untreated pernicious anemia revealed stimulation for the growth of Lactobacillus lactis equivalent to 0.3 to 1.8 mcg. vitamin B_{12}/Gm. of dried feces. This indicates a daily fecal output of vitamin B_{12} by patients with pernicious anemia many times greater than that necessary to produce remission when introduced parenterally This suggests that in pernicious anemia the absorption of vitamin B_{12} derived either from dietary sources or by intestinal bacterial synthesis may be defective.

University of Michigan
Ann Arbor Mich.

203. RAVINA, A. *Un progrès décisif de thérapeutique anti-anémique la vitamine B_{12} (Conclusive progress in antianemic therapy vitamin B_{12})* Presse méd. 57: 89-90, Jan. 22, 1949

American and English articles regarding vitamin B_{12} are reviewed.

204 SPIES, T D., SUAREZ, R. M., GARCIA LOPEZ, G., MILANES, F., STONE, R. E., LOPEZ TOCA, R., ARAMBURU T., and KARTUS S. *Tentative appraisal of vitamin B_{12} as a therapeutic agent,* J.A.M.A. 139 521-525, Feb. 19 1949

Vitamin B_{12} was used in the treatment of 21 patients with macrocytic hyperchromic anemia. Of these, 4 had nutritional macrocytic anemia, 1 had nontropical sprue, 11 had tropical sprue, and 5 had pernicious anemia. The vitamin was given intramuscularly and the total dose ranged from 4 to 25 mcg. The administration of vitamin B_{12} was followed by excellent clinical and hematologic improvement in all 21 patients. They felt stronger and their appetites returned four or five days after the vitamin was injected. The patients with sprue noted a decrease in abdominal distension and in the volume of the stools after six or seven days.

Vitamin B_{12} brought about improvement in 14 patients with pernicious anemia. Nine of these had severe and 5 had mild subacute combined degeneration. Within two weeks after the administration of vitamin B_{12} the peripheral nerve and posterior column involvement was greatly improved in these patients

Several additional patients also were treated with vitamin B_{12}. One had both pernicious anemia and sprue, and the symptoms of both diseases subsided after oral treatment with 25 mcg. vitamin B_{12} which had been incubated with gastric juice obtained from a patient with duodenal ulcer This patient had previously failed to respond to treatment with 50 mcg. vitamin B_{12} concentrate orally Apparently the human gastric juice is able to effect the utilization of vitamin B_{12}.

205 SPIES T D., STONE, R. E., GARCIA LOPEZ, G., LOPEZ TOCA, R., and ARAMBURU T *Observations on vitamin B_{12} as an antianemic substance* Internat. Ztschr f. Vitaminforsch. 21 328-340 1949

206. STURGIS C. C. *Advances in our knowledge concerning the etiology and treatment of hematological disorders,* Bull. New York Acad. Med. 25 84-99 Feb. 1949

In anemia due to chronic infection, penicillin, streptomycin, or the sulfonamide drugs should be employed either alone or in combination with surgical measures. Folic acid may be of value in the treatment of sprue, the macrocytic anemias of pregnancy nutritional macrocytic anemia, and anemia due to intestinal disturbences, but it is without beneficial effect on the neurologic lesions of pernicious anemia. Improvements in blood picture and neurologic manifestations have been induced with vitamin B_{12}. It may be possible to substitute vitamin B_{12} for liver extract therapy in patients who develop allergic reactions to the latter

Also discussed are treatment recommendations in agranulocytosis, Hodgkin s disease, chronic myelogenous and lymphatic leukemias.

University of Michigan
Ann Arbor Mich.

207 STURGIS C. C. *The treatment of hematologic disorders,* Postgrad. Med. 5 300-306, April 1949

This is a general article on the treatment of hematologic diseases, including pernicious anemia, Hodgkin s disease, leukemia, and polycythemia.

The author has treated 6 cases of pernicious anemia with vitamin B_{12}. The dose usually used was 1 microgram per day but 2 of the patients received only 0.45 mcg. per day and showed a definite, though substandard response.

208. SPIES, T D., GARCIA LOPEZ, G., MILANES F., LOPEZ TOCA, R., and ARAMBURU T *A note on the oral versus parenteral administration of vitamin B_{12}.* South. M. J 42 528-531, June 1949

The effect of vitamin B_{12} administered orally was studied in 4 patients with pernicious anemia, 4 with tropical sprue, and 4 with nutritional macrocytic anemia. All 12 patients were in relapse, and all had macrocytic hyperchromic anemia, a red blood cell count of 2,600,000 or less, a color index of 1.0 or more, and megalol arrest of the sternal bone marrow These patients

hospitalized and kept on a diet devoid of meat and meat products until baseline determinations of blood cell counts, hemoglobin, and reticulocyte counts had been made. Vitamin B_{12} was then administered orally. After four or five days there was general clinical improvement, reticulocytosis reached a peak around the seventh day and subsequently there was an increase in red blood cells and hemoglobin. The megaloblastic arrest of the bone marrow tended to disappear and the marrow became more normal in appearance. Because of scarcity of material the response obtained was somewhat submaximal. In general, thirty to fifty times as much vitamin B_{12} is required when given orally as when given by injection. If the vitamin is incubated with normal human gastric juice, however, only five to ten times the parenteral dose is required. Responses were obtained from 10 or 30 mcg. intramuscularly or 300 mcg. orally.

In 2 of the patients with pernicious anemia, combined system disease developed despite treatment with 30 mcg. vitamin B_{12} by mouth. When 30 mcg. was incubated with human gastric juice and administered orally to these patients, the blood responded and the acute signs of combined system disease disappeared. Parenteral administration has the advantages of requiring less material and of permitting more accurate supervision.

Northwestern University
Chicago, Ill.
Hillman Hospital
Birmingham, Ala.
Calixto Garcia Hospital
Havana, Cuba

209 SPIES, T. C., GARCIA LOPEZ, G., MILANES, F., STONE, R. E., LOPEZ TOCA, R., ARAMBURU T., and KARTUS, S.: *Observations on the effect of an animal protein factor concentrate on persons with the macrocytic anemia of pernicious anemia, of nutritional macrocytic anemia and of sprue and on persons with nutritional glossitis.* Blood 4: 819-826, July 1949.

Animal protein factor concentrate produced a hematologic response in each of 5 cases of pernicious anemia in relapse, 4 cases of nutritional macrocytic anemia in relapse, and 3 cases of tropical sprue in relapse. It also caused the redness and soreness of the tongue to disappear in 3 cases of nutritional glossitis *not* associated with anemia. The concentrate, a product of microorganisms, was administered by intramuscular injection in dosages ranging from a total of 5 cc. over a period of 23 days to 5 cc. daily for 14 days. Representative cases are described briefly. In the case illustrating the response of pernicious anemia to animal protein factor there was no objective evidence that neurologic symptoms were improved although there was general symptomatic improvement. Treatment had to be discontinued in the case of glossitis described because of local hypersensitivity to the concentrate.

Northwestern University
Chicago, Ill.

210. JONES, E., DARBY, W. J., and TOTTER, J. R.: *Pernicious anemia and related anemias treated with vitamin B_{12}.* Blood 4: 827-844, July 1949.

Study of the hematologic data on 8 patients with anemia who were treated with vitamin B_{12} for periods up to six months indicates that a total dosage averaging less than 0.75 mcg. daily when the vitamin is given parenterally and at intervals of several days, will not establish and maintain as high blood levels as will adequate liver therapy. All of the patients responded hematologically to vitamin B_{12} and 2 who had mild neurologic symptoms improved. Parenteral daily doses of 1.0 mcg. promoted good erythropoiesis in one patient, but erythropoiesis seemed to be more consistently rapid in patients who received 2.5 mcg. or more daily. It would appear that the average initial daily dose should be about 3.0 mcg. in order to obtain a maximum rate of hemopoiesis. The observations in these cases again emphasized the unreliability of the reticulocyte count as a quantitative measure of the activity of an anti-pernicious anemia agent or of the adequacy of therapy in a given patient. Vitamin B_{12} caused a decrease in fecal urobilinogen, an effect that has been observed by other investigators using liver extract for pernicious anemia and by the authors following administration of folic acid for this disease. No effect of vitamin B_{12} on urinary pteroylglutamic acid (PGA) or porphyrin excretion could be detected.

The hematologic response of a patient with nutritional macrocytic anemia to vitamin B_{12} was equal to the responses that had previously been obtained in this patient with liver extract, and there was concomitant symptomatic improvement. A patient with sprue responded to the vitamin hematologically but after receiving large amounts over a period of 27 weeks his erythrocyte count had not equalled that previously reached with liver extract or folic acid. A hematologic relapse had occurred, however, while the patient was receiving presumably adequate amounts of folic acid. This fact and the incomplete response to vitamin B_{12} suggest that hemopoietic factors in addition to folic acid and B_{12} may be required by some patients for a maximum erythrocytic response. The absorptive defect in a patient with anemia associated with intestinal lipodystrophy was not altered by administration of vitamin B_{12}.

211 DARBY, W. J., and JONES, E.: *Observations on two newer hemopoietic vitamins—vitamin B_{12} and animal protein factor.* Am. J. Med. 7: 241, Aug. 1949 (in Soc. Proc.)

The responses to crystalline vitamin B_{12} administered parenterally to 11 patients with pernicious anemia, nutritional macrocytic anemia, or sprue indicate the approximate equivalence of 1 microgram of vitamin B_{12} to 1 U.S.P. unit of anti-pernicious anemia liver extract. In pernicious anemia, the remissions produced by B_{12} have been characterized by relief of glossitis, an increased sense of well-being, an initial hemopoietic response, maturation of the megaloblastic marrow, weight gain, decrease in fecal urobilinogen and, in 2 patients, disappearance of early neurologic symptoms. In 2 cases of sprue, the results have been less clearly defined, and a greater quantitative requirement for B_{12} in this syndrome is probable. Nutritional macrocytic anemia has responded in a manner comparable to pernicious anemia. Evidence of approximate correspondence of the activity of the animal protein factor in patients with pernicious anemia (parenteral administration) and in the chick, has been found. Since these two factors are effective parenterally it does not appear that they correspond to Castle's extrin-

sic factor although their association with animal protein would imply that they may

Vanderbilt University School of Medicine
Nashville, Tenn.

212. SPIES T D., STONE, R. E., GARCIA LOPEZ, G., MILANES, F., and LOPEZ TOCA, R.. *Vitamin B_{12} by mouth in pernicious and nutritional macrocytic anaemia and sprue,* Lancet 2 454-456, Sept. 10, 1949

Illustrative cases are summarized to indicate the effect of oral vitamin B_{12} therapy in pernicious anemia, nutritional macrocytic anemia, and tropical and nontropical sprue. The results reported show that such treatment if adequate is often effective. Most patients, however respond slowly to oral B_{12} even when given in 30 to 60 times the required parenteral dose. The authors conclude that vitamin B_{12} should always be administered parenterally if there is severe cardiac failure, severe neural degeneration, or severe alimentary disturbance, and at least initially in severe cases of megaloblastic anemia. Although an allergic reaction to parenteral vitamin B_{12} has not yet been encountered, this is considered a possibility in which case oral treatment may be useful. The minimum, maximum, and optimum doses of vitamin B_{12} vary with the patient and also from time to time in the same patient, and therefore no set routine of treatment can be followed satisfactorily

Northwestern University Medical School
Chicago, Ill.
Hillman Hospital
Birmingham, Ala.
University of Havana
Havana, Cuba

213. BOEHRER, J J: *Recent advances in pernicious anemia, hypochromic anemias and hemolytic anemias,* Minnesota Medicine 32 889- Sept. 1949

214 CHEYMOL, J: *Vitamin B_{12} and antipernicious anemia factor* Semaine d. hôp. Paris 25 3305- Oct. 30, 1949

215. STURGIS, C. C. *Recent advances in treatment of hematologic disorders* J.A.M.A. 141: 969-978, Dec. 3, 1949.

This review article states that liver extract given intramuscularly is the treatment of choice for pernicious anemia, but vitamin B_{12} may prove to be equally effective when administered intramuscularly or perhaps orally with intestinal extract. Folic acid intramuscularly or orally is highly effective for all varieties of macrocytic anemia except pernicious anemia, and is the most satisfactory treatment for the macrocytic anemia of pregnancy in which liver extract and vitamin B_{12} are often, if not always, ineffective.

Therapy in myelogenous leukemia, Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma is also discussed

University of Michigan
Ann Arbor Mich.

216. SMITH, E. L.: *Folic acid, vitamin B_{12} and anemia,* Nature 164 986-987 Dec. 10, 1949

This is a review of a symposium on folic acid, vitamin B_{12} and anemia. The papers read present a survey of the historical development of liver and related therapy of anemia, and of the work on folic acid and vitamin B_{12} and summarize present day's chemical, biochemical and clinical results.

217 UNGLEY C. C. *Recent work on vitamin B_{12},* Lancet 1 353-354, Feb. 25, 1950 (in Soc. Proc.)

The action of vitamin B_{12} and some kindred substances isolated by Smith was studied in two groups of cases idiopathic addisonian anemia with gastric atrophy and permanent loss of Castle's intrinsic factor which leads to deficient absorption of B_{12} and nonaddisonian megaloblastic anemias such as those associated with pregnancy and intestinal disorders.

When an initial dose of 5 to 80 mcg. was given, the increase of red blood cells on the fifteenth day was proportional to the logarithm of the dose. Up to the fifth day there was little effect with any of these single doses, and after the fifteenth to twentieth day the red cell curve flattened. It was found that for maintenance, in the absence of subacute combined degeneration of the cord, 10 mcg. every two weeks was sufficient over periods up to 18 months.

Of 8 patients receiving B_{12} during periods of 9 to 19 months, 4 showed improvement greater than that expected with liver extract, 2 showed improvement as great, and 2 showed less. The author concluded that B_{12} was as effective as liver extract in addisonian anemia as well as in subacute combined degeneration of the cord. Weekly doses of 40 mcg. were usually sufficient in the first six months, with 80 mcg. weekly for maintenance.

Twenty patients with addisonian pernicious anemia received Smith's "unnamed crystalline substance," obtained from Streptomyces griseus. The initial doses were as effective as B_{12} with regard to blood picture, glossitis and neurologic symptoms. One patient with apparent addisonian anemia, who had been treated six months previously for syphilis, showed poor response to B_{12} 2.5 mg. of folic acid daily (total, 15 mg.) caused a satisfactory reticulocyte rise. A subsequent 10 mcg. of B_{12} produced a renewed rise in the red cell level which had begun to fall.

Oral administration of B_{12} (or of the "unnamed substance") gave poor results a single 5 mcg. injection was more effective than 1,920 mcg. given orally over 24 days. However when 50 cc. of normal unfiltered gastric juice was added to a 5 mcg. daily oral dose of B_{12} a satisfactory response was obtained, but it still required 50 mcg. of B_{12} orally to equal the effect of 10 mcg. of B_{12} by parenteral administration. When the gastric juice was passed though Seitz filters, the intrinsic factor activity was removed.

In 2 of 3 cases of megaloblastic anemia with intestinal disorders, much larger (4 to 14 times) doses of B_{12} were necessary to obtain response than in cases of addisonian anemia. One of the 3, who did not respond to B_{12}, showed favorable results on receiving folic acid.

Six patients with megaloblastic anemia of pregnancy which were refractory to 65 to 80 mcg. doses o showed excellent response to 2.5 mg. of folic aci

In referring to the problems still to be solved the author noted that yeast given orally could cause a remission in pernicious anemia. If gastric juice was given adjunctively one-tenth of the dose sufficed this dose was also sufficient in nonaddisonian anemias. Yeast extracts apparently contain no B_{12} yet they seemed to be a source of the extrinsic factor

218. SPIES T D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and REBOREDO A.: *Anti anemic properties of a reaction product of vitamin B_{12} and the intrinsic factor* South. M. J 43 206-208, March 1950.

The authors experiences with capsules of Berubozyme suggest that the time when macrocytic anemia can be treated with a single capsule per day is near at hand. Each of these capsules contains a reaction product of 9 mcg. of vitamin B_{12} and the intrinsic factor present in 0.33 Gm. of concentrate of hog duodenum. Four patients with macrocytic anemia in relapse (1 with pernicious anemia, 2 with tropical sprue, and 1 with nutritional macrocytic anemia) were treated for 10 days, and each showed some clinical and hematologic improvement. The reticulocytes reached a peak nine to 11 days after treatment was begun. None of the cases gave a maximum response, which indicates that a larger dose is required if treatment is continued no longer than 10 days, but supplies were not available for this purpose.

Hillman Hospital
Birmingham, Ala.
General Calixto Garcia Hospital
Havana, Cuba

219 CAMERON D G *Therapy of the blood diseases,* Mod. Med. 18 61-64, 142-156, April 15, 1950.

This review contains references to liver extract in the treatment of pernicious anemia, and to the use of vitamin B_{12} for the same purpose. Given orally vitamin B_{12} acts like the "extrinsic" factor and given parenterally it acts like the "anti-pernicious anemia" factor The oral administration of 5 mcg. of vitamin B_{12} daily has no effect on the blood in pernicious anemia when given alone, but produces a maximal response when given with 50 to 150 cc. of normal gastric juice. This vitamin appears to relieve and to prevent subacute combined degeneration of the spinal cord, and it may eventually replace liver extracts in the treatment of pernicious anemia, although liver extract must be considered the treatment of choice until the value of vitamin B_{12} is finally established.

Folic acid relieves all types of macrocytic anemia associated with megaloblastic bone marrow but it does not relieve or prevent neurologic complications in pernicious anemia and may even precipitate them. It can be given parenterally or orally It is the treatment of choice in pernicious anemia of pregnancy in which liver extract and vitamin B_{12} are often ineffective.

Since leukemia cells contain folic acid which may be necessary for their growth, folic acid antagonists such as aminopterin may produce short remissions in leukemia. The drug is given intramuscularly or orally in daily doses of 1 mg. until toxic symptoms appear and is then continued in smaller amounts.

Urethane has also been used in leukemia, and is effective in the chronic myeloid type.

Montreal General Hospital
Montreal, Canada

220. GOLDSMITH, G.: *Vitamin B_{12}*, Lancet 2 407 Sept. 23, 1950 (in Soc. Proc.)

"Dr Grace Goldsmith (U.S.A.) thought that 4 µg. (of vitamin B_{12}) injected daily gave results poorer than liver extract and reported failure with oral treatment, though in sprue and nutritional macrocytic anaemia 5-30 µg. by mouth daily did evoke some response. She recalled that a good haemopoietic response has been obtained in pernicious anaemia when 5 µg. of B_{12} is given orally together with a duodenal extract but she did not mention what was the effect of the duodenal extract alone. Finally she suggested that vitamin B_{12} may not maintain pernicious-anaemia patients at proper blood levels for long periods."

221 OWREN P A.: *Vitamin B_{12}*, Lancet 2 407 Sept. 23, 1950 (in Soc. Proc.)

"In the discussion Prof. P A. Owren (Norway) produced more evidence that vitamin B_{12} is not the anti anaemic principle and that responses to it are partial. He showed that the prothrombin concentration is low in untreated pernicious anaemia and remains low when B_{12} treatment has raised the haemoglobin; folic acid has the same effect, but some liver extracts will restore the picture. He also pointed out that abnormal haemoglobin of the foetal type remains, and macrocytosis persists, with B_{12} treatment."

222. SPIES, T D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and REBOREDO A.: *Folic acid and vitamin B_{12} in anemia,* GP 2 37-43, Sept. 1950.

An intensive two-year study of selected cases of anemia indicates that folic acid and vitamin B_{12} should be used together in practical everyday therapy One case history is given which is representative of 3 patients with macrocytic or pernicious anemia of pregnancy who responded to folic acid. In a second illness, the patient failed to respond to vitamin B_{12} until folic acid was added, whereupon rapid improvement began. She continued well under maintenance treatment with folic acid.

A second case report is representative of 3 patients who responded hemopoietically and clinically to folic acid but required vitamin B_{12} to relieve neurologic symptoms. Maintenance treatment consisted of an injection of 30 mcg. vitamin B_{12} twice a month. A third case is illustrative of 10 elderly patients with pernicious anemia who had been treated for 20 years or more. Their blood levels remained low during treatment with 150 to 250 mcg. vitamin B_{12} a week but became normal when folic acid was added. Maintenance therapy with 60 mcg. of vitamin B_{12} a week and 10 mg. of folic acid daily was effective. Since both folic acid and vitamin B_{12} have specific functions as well as functions in common, the giving of both for complete and continued hemopoiesis in properly selected patients is recommended.

Hillman Hospital
Birmingham, Ala.
General Calixto Garcia Hospital
Havana, Cuba

223. WOODS, D D: *Folic acid vitamin B_{12} and anemia*, J Pharm. Pharmacol. 2 537-540, Sept. 1950.

A review; 27 references.

224 BARNARD R. D: *Splenectomy for blood dyscrasias* Mod. Med. 18 16, Oct. 1, 1950 (In Correspondence)

Oral doses of Streptomyces-derived antibiotics (aureomycin, chloromycetin, streptomycin, and some not yet characterized) in quantities just sufficient to suppress the ordinary proteolytic intestinal flora and to substitute a predominantly saccharolytic gram-positive flora have been observed to cause such rapid clinical and hematologic improvement in 8 successive patients with hemolytic anemia that the writer thinks splenectomy for this disease may soon be discarded.

Work in progress indicates that peptones, proteoses, arising from proteolytic activity of the flora in an intestinal tract of heightened permeability may be responsible for many hemopoietic arrests. Circulating peptone may be curtailed by intestinal coliform organism suppression (by oral antibiotics) by altering intestinal permeability and the pathway of protein catabolism (corticotherapy) or by bolstering cholinesterase synthesis (B_{12} and folic acid)

These effects may overlap, that is, oral antibiotics or folic acid may cause salt and water retention indistinguishable from that of corticotherapy folic acid and B_{12} have been found to have some coliform-suppressor antibiotic activity

Terramycin has produced two complete remissions in infantile megaloblastic anemia. The daily dose was 150 mg.

Radiation anemia and that of glomerulonephritis both appear to be more responsive to minimal oral antibiotic therapy than to any other agency not excluding transfusion.

These results would seem to warrant a trial of oral antibiotic therapy in refractory anemias before resorting to splenectomy in cases which are not urgent.

Lawrence, L.I., N.Y.

225. APPEL, W: *Rationale of therapy of blood diseases*, J Michigan State M. Soc. 49 1188-1190, 1204 Oct. 1950.

The role of vitamin K in clotting is discussed in an outline of the clotting mechanism. Therapeutic administration of large quantities of vitamin K is indicated when excessive amounts of dicumarol have been given to a patient. Vitamin B_{12} is mentioned as containing the erythrocyte maturing factor as does folic acid, but B_{12} is apparently able to prevent and control nervous system involvements. Folic acid antagonists seem to be of most value in acute leukemia. HN_2 does not produce satisfactory remissions. HN_2 in chronic myelogenous leukemia produces temporary palliative results by damaging susceptible cells. Use of too large doses of HN_2 in chronic lymphatic leukemia may damage hemopoietic tissue.

Kalamazoo, Mich.

226. SPIES T D., GARCIA LOPEZ, G., MILANES F., LOPEZ TOCA, R., REBOREDO A., and STONE, R. E.: *The response of patients with pernicious anemia, with nutritional macrocytic anemia and with tropical sprue to folinic acid or citrovorum factor* South. M. J 43 1076-1082, Dec. 1950.

227 WESTERMAN B. D *Folic acid and vitamin B_{12} as antianemia factors* J Home Econ. 42 199-203, 1950.

A brief review of the literature on investigations leading up to the discovery and isolation of folic acid and vitamin B_{12}.

228. BARNARD R. D., CARACAPPA, J M., SCHWARTZ, M., GORDON G B., and MOLDOVER, A. *Further observations on the response of erythroblastic, megaloblastic, and radiation anemias to Streptomyces-derived "animal protein factor" and antibiotics*, New York State J Med. 51 1739-1745 July 15 1951

Previously reported work (Barnard and Fox: Ohio State M. J 46: 784, 1950 [Abstr 325]) has shown that a crude Streptomyces griseus residue concentrate, marketed as "animal protein factor" for fortification of animal feed, has an arresting or reversing action on anemia in patients with acute leukemia. This action appears to be due to some hematinic factor other than B_{12}, since it is not produced by more refined preparations of this vitamin. Preparations from species of Streptomyces which do not produce antibiotics did not give comparable results, which indicates that the hematinic effect may be due to the antibiotic content of the effective preparations.

Streptomyces griseus concentrate is a by-product of streptomycin manufacture which, unlike streptomycin but like *terramycin, aureomycin* and chloramphenicol, is effective by oral administration. It produces systemic effects in lower doses than do these antibiotics, and these effects are not attributed to bacteriostatic action although the bacteriostatic action of minute oral doses of antibiotics is also present, acting by changing the fecal bacteria from a predominantly gram-negative proteolytic to a gram-positive saccharolytic type.

Seven case histories are presented and summarized as follows "A crude Streptomyces griseus residue was found to have marked hematinic effects similar to those exhibited by adrenocorticoids in hemopoietic suppressive conditions such as erythroblastic arrest (acquired hemolytic) anemia, megaloblastic anemia, and radiation myelopathy This effect could not be attributed to the B_{12} content of the residue, and two apposed cases in each category are presented to show that the same effect can be produced by a coliform-aerogenes suppressor antibiotic. It is postulated that ingestion of such an antibiotic, both by direct depressant effect on intestinal proteolytic organisms and by the indirect implantation and maintenance of a saccharolytic flora, may limit the absorption of cholinergic proteoses. An enhanced production of erythrocytic cholinesterase is thereby permitted, and the latter may instigate accelerated hemopoiesis. At time, the possibility that hemopoiesis may be

substances derived from the non-sporulating gram-positive intestinal organisms which flourish during this therapy is not ruled out. Whatever the mechanism, the hematinic effect of sustained, small, oral antibiotic dosing holds promise of wide clinical application, not only to the management of the myelosuppressive hematologic dyscrasias, but to all allergic, atopic and adaptative diseases as well.

"Treatment failures with this modality seem to have occurred predominantly where intestinal coliform suppression did not take place or where intolerance to the implanted saccharolytic flora developed. In some instances, profound adrenocorticomimetic effects, among which salt and water retention were pronounced, forced an abandonment of the antibiotic dosage schedule."

A footnote states that sodium and water retention due to protracted therapy with terramycin or the Streptomyces animal protein factor must now be emphasized because about one of seven patients on such therapy develops gross edema which responds to theophyllin-ammonium chloride diuresis. It is believed that 3 leukemic patients with diminished cardiac reserve died from congestive failure before the corticoid features of this type of therapy were recognized. In two other instances, symptoms resembling those of Cushing s syndrome appeared.

Lawrence, N. Y. (R.B.B.); Elmont, N. Y. (U.M.C.); Rosedale, N. Y. (M.S.) and Brooklyn, N. Y. (L.B.C. and A.M.)

229 LIMARZI, L. R. *Treatment of blood dyscrasias,* Postgrad. Med. 10 51-56, July 1951

Among the subjects discussed in this review is the use of vitamin B_{12} in pernicious anemia and some other macrocytic anemias. Megaloblastic anemia of pregnancy is said to respond well to folic acid and liver extract, but not to vitamin B_{12}. The best treatment for megaloblastic anemia of infancy and childhood may be 200 mg. of ascorbic acid, 15 mg. of folic acid, and injectable crude liver extract given intramuscularly at first and then orally

Included in the article are discussions of therapy in Hodgkin s disease, chronic myeloid leukemia, multiple myeloma, polycythemia vera, and agranulocytosis.

230. EDITORIAL: *Vitamins and blood regeneration,* J.A.M.A. 147 979-981, Nov 3, 1951

In this editorial, pertinent literature on the effects of vitamins, particularly vitamin B_{12} and folic acid in various anemias, is discussed, 13 references being cited.

231 GIRDWOOD R. H.: *Reports of societies vitamin B_{12} therapeutic value,* Brit. M. J 1 161, Jan. 19 1952.

" Dr R. H. Girdwood dealt with the complicated interrelationships between vitamin B_{12}, folic acid, and folinic acid both in the nutrition of micro-organisms and in the cure of anaemia. He concluded that both vitamin B_{12} and folic acid were necessary for normoblastic blood, and that folic acid was probably converted in the body to folinic acid. Other factors, such as ascorbic acid and the anti-anaemic factor of Wills, were also important, and the picture might be further complicated by the presence of inhibitory factors. "

232. UNGLEY C. C. *Reports of societies vitamin B_{12} therapeutic value,* Brit. M. J 1 161 Jan. 19 1952.

" Dr C. C. Ungley discussed the value of vitamin B_{12} in the treatment of various forms of anaemia, and explained that it was only effective when the bone marrow was megaloblastic. Not all forms of megaloblastic anaemia, however responded to therapy Thus the anaemias of pregnancy and the puerperium, as observed in temperate climates, failed to respond to vitamin B_{12} but were corrected by folic acid. The so-called 'tropical sprue' sometimes responded to vitamin B_{12}. Anaemia associated with cirrhosis of the liver was seldom megaloblastic, but, when it was, vitamin B_{12} might be effective. Anaemia after total gastrectomy had been corrected by folic acid, but not by vitamin B_{12}. Anaemia produced by a fish tapeworm had been cured either by vitamin B_{12} or by the expulsion of the worm the parasite had been found to be rich in vitamin B_{12}, and presumably caused anaemia by absorbing supplies otherwise available to its host.

"Dr Ungley emphasized that, while vitamin B_{12} could correct not only the blood picture in pernicious anaemia but also the glossitis and nervous lesions, folic acid could only temporarily correct the blood picture. Doses of 1 µg. often produced definite improvement, but when patients had nervous lesions or suffered from intercurrent diseases 100 µg. monthly should be allowed. "

233. PATEL, J C. *Necessity of B-complex factors in the treatment of anemias* J Indian Med. Assn. 21 191 194 Feb. 1952 (abstr Am. J Digest Dis. 19 235, July 1952)

"In the various anemias seen in India, Patel has found it best to rely upon liver extract, folic acid or vitamin B_{12} without any additional substances. In iron deficiency he uses only iron. He does not believe that the various factors in B-Complex, singly have any effect on human anemia. Used in combination, in anemias, they may upset the enzyme equilibrium of the body. Liver extract, crude or refined, still remains a potent method of avoiding this accident while, at the same time, improving the anemia."

234 HECK, F J: *Proper use of iron, liver extract, vitamin B_{12}, and folic acid in anemias* J.A.M.A. 148 783-788, March 8, 1952.

Author s summary "Except possibly in some very unusual situation the treatment of anemia resolves itself into the use of specific substances depending on the morphologic blood picture. In hypochromic anemias, which are usually due to loss of blood, the proper treatment is the oral use of a preparation of iron. For these anemias, liver extract, vitamin B_{12}, folic acid, desiccated hog stomach, and stomach-liver or iron-stomach-liver preparations serve no useful purpose and are definitely not indicated. In those unusual instances in which iron taken by mouth cannot be tolerated or produces an increase in gastrointestinal symptoms, it can be given intravenously

"The commonest diagnosis of macrocytic anemias with megaloblastic type of bone marrow in northern United States is pernicious anemia. The most desirable form of treatment of this type of anemia is the parenteral use of a potent liver extract or the parenteral use of vita

min B_{12}. Preparations of liver or stomach or liver-stomach combinations for oral administration can be used and are effective when given in adequate amounts. The presence of neurologic manifestations with pernicious anemia requires that much larger quantities of potent material be used either parenterally or orally Iron is not indicated unless loss of blood is associated with pernicious anemia. Folic acid, while not contraindicated, is not necessary in the complete treatment of pernicious anemia and when used alone, constitutes inadequate treatment. " (Experience with citrovorum factor is insufficient as yet for anyone to know whether it will merely react on a megaloblastic marrow and convert it to normal and then, as in the case of folic acid, fail to prevent gastrointestinal and neurologic manifestations and permit eventual relapse of hematologic symptoms. The author's limited experience with citrovorum factor confirms the good hematologic response recently reported.)

"Concerning other diseases associated with megaloblastic anemia, there are differences of opinion regarding the response to vitamin B_{12} and folic acid. Some patients will respond only partially or not at all to vitamin B_{12}, and subsequently a good result can be obtained with folic acid.

"None of the substances discussed in this paper are of value in the treatment of the anemia associated with (1) various types of malignant lesions, (2) diseases of the reticuloendothelial system, and (3) diseases in which there is toxemia or interference with metabolism of hemoglobin.

"Treatment with iron, liver extract, vitamin B_{12}, or folic acid is of benefit only when these substances are deficient, and none of these forms of medication is helpful in anemia due to some cause other than that produced by these specific deficiencies. A useful maxim then is: Determine the type of anemia present, give only the medicament indicated do not mix the treatment." Fifteen references are cited.

Mayo Clinic
Rochester Minn.

235 REISNER, E. H., Jr. *The pathogenesis of anemia as a basis for rational treatment*, New York State J Med. 52 1645-1648, July 1, 1952.

An initial hemogram greatly facilitates diagnosis of the type of anemia. If the bone marrow is not functioning, the color index will suggest the cause. Nutritional macrocytic anemia and sprue respond to vitamin B_{12} or folic acid. In pernicious anemia, folic acid apparently speeds up reactions that exhaust the already depleted stores of vitamin B_{12} and may aggravate the neurologic lesions of the disease therefore vitamin B_{12} should be used in such cases. Megaloblastic anemia of infancy and pernicious anemia of pregnancy respond best to folic acid.

New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N. Y.

236. UNGLEY C. C. *Clinical and laboratory aspects of vitamin B_{12}*, Lancet 2 134, July 19 1952 (in Soc. Proc.) *Vitamin B_{12}*, Brit. M. J 2 154 July 19 1952 (in Soc. Proc)

It is essential to differentiate between megaloblastosis due to lack of vitamin B_{12} and that due to lack of folic acid. Anemias involving dietary deficiency have been benefited by vitamin B_{12} therapy A case is cited in which a patient with anemia subsequent to a total gastrectomy responded well to oral administration of 1,000 mcg. of the vitamin.

The fish-tapeworm anemia of Finland has been attributed to some toxin in the worm; its dried body however has been found to be "a rich source of vitamin B_{12}," and was curative when orally administered This observation suggests that the living worm takes up vitamin B_{12} in the intestine.

Newcastle-upon-Tyne, England

237 RHEINGOLD J J: *The treatment of the anemias* M. Ann. District of Columbia 21 368-371, July 1952.

The need for accurate diagnosis of the cause of anemia is stressed. Anemia resulting from deficiency of the erythrocyte maturing factor or the so-called anti-pernicious anemia factor is treated with refined liver extract, vitamin B_{12}, or folic acid. In pernicious anemia, liver extract or vitamin B_{12} is necessary to prevent the development of neurologic symptoms, and combining such treatment with folic acid does not seem to produce better results. In sprue, however combined therapy with liver or B_{12} and folic acid seems to give better results than does either of these agents alone. Pernicious anemia of pregnancy and of infancy seem to respond better to folic acid than to either vitamin B_{12} or liver extract. Atypical megaloblastic anemia responds sometimes to one and sometimes to another agent in some instances a combination may be necessary

The recommended dosage of vitamin B_{12} in pernicious anemia-like diseases is 30 mcg. a day intramuscularly for a patient whose red blood cell count is approximately 1,000,000. When a maximum reticulocyte response has been gained, this dose may be given every other day and later at longer intervals until eventually an injection of 30 mcg. once a month will suffice.

In anemias involving disturbance of the bone marrow supportive therapy is helpful, and occasionally corticotropin or cortisone will result in the formation of normal bone marrow When splenomegaly is involved, splenectomy is usually the treatment of choice. In cases where there is no response, corticotropin or cortisone may be helpful in obtaining a remission. These hormones have also been used successfully by some investigators in acquired hemolytic anemia, and the author believes they should be tried before splenectomy is resorted to.

Iron deficiency anemias, and anemias resulting from blood loss or destruction are also discussed.

George Washington University School of Medicine
Washington, D. C.

238. BETHELL, F H.: *Treatment of anemia*, Wisconsin M. J 51 1082-1084, Nov 1952.

Accurate classification as to type of anemia is a primary requisite. Complex mixtures of vitamins, other hematinics as proprietary preparations

have no place in treatment. Specific therapy includes measures to supply hemopoietic factors which may be missing (iron, folic acid, vitamin B_{12}, ascorbic acid, dietary amino acids) and to diminish excessive rate of blood destruction.

Microcytic hypochromic anemia is treated with iron-containing foods or iron orally or intravenously

The pathogenesis of macrocytic anemias has been elucidated in recent years. It is now known that both folic acid and vitamin B_{12} are required for normal hemopoiesis and that they participate in widespread metabolic processes. Folic acid is widely distributed in foods, but chiefly in conjugated forms that require enzymatic action for the release of the free vitamin, which is believed to be the only form that can be absorbed. Thus, folic acid deficiency may result from failure of conjugase action. Evidence that the biologically active form of folic acid is folinic acid has been obtained ascorbic acid facilitates the conversion of folic to folinic acid in living tissues. Ascorbic acid plays a part in hemopoiesis both in metabolism of folic acid and in utilization of iron. Appreciable amounts of vitamin B_{12} may become available through bacterial synthesis, but intestinal bacteria that require an exogenous source of this vitamin for growth may utilize it to the deprivation of the host. Intrinsic factor may combine with the extrinsic factor (vitamin B_{12}) forming a microbiologically inactive complex; this may facilitate absorption, perhaps by preventing utilization by coliform organisms. In pernicious anemia the primary factor appears to be lack of intrinsic factor resulting in a conditioned vitamin B_{12} deficiency Dietary lack of folic acid appears to be a more common cause of nutritional macrocytic anemia than lack of B_{12}. Chronic intestinal disorders (idiopathic steatorrhea, disseminated ileitis, partial obstruction, diminished absorptive surface) may impair resorption of both folic acid and B_{12}, resulting in the neurologic changes seen in true pernicious anemia.

Specific measures available for treatment of macrocytic megaloblastic anemias include liver extract, desiccated stomach, vitamin B_{12} and folic acid. Vitamin B_{12} usually constitutes a complete form of replacement therapy for pernicious anemia for macrocytic megaloblastic anemias other than pernicious, folic acid orally (parenterally if intestinal disease is present) or B_{12} orally if pernicious anemia can be excluded, will usually correct dietary deficiency of these vitamins.

In hemolytic anemias the treatment used in anemia caused by defective blood formation is ineffective. Corticotropin and cortisone have proved of real merit in idiopathic acquired hemolytic anemia they may terminate an acute crisis, permit reasonably safe splenectomy or even induce long-lasting complete remissions without other therapy or need of continuous administration of the hormones.

University of Michigan
Ann Arbor Mich.

239 LUTTGENS, W F *The newer hematinics, their use and abuse,* California Med. 77 369-373, Dec. 1952.

In a review based on 51 published articles the author discusses the action of vitamin B_{12}, folic acid, citrovorum factor and saccharated oxide of iron given intravenously Vitamin B_{12} appears to be the active principle of refined liver extract and is effective in pernicious anemia when given alone parenterally or with a source of intrinsic factor such as normal gastric juice, by mouth. Folic acid usually brings about complete remission in the other types of megaloblastic anemia, and these remissions may be permanent notwithstanding cessation of therapy

Because folic acid can obscure changes in the blood and thus prevent early recognition of pernicious anemia, in turn permitting neurologic disease to develop, inclusion of folic acid in polyvitamin preparations is decried. Saccharated iron is relatively safe for intravenous administration but is of value only in iron-deficiency hypochromic anemia, and is seldom indicated. Overdosage should be avoided because the body cannot excrete iron.

Stanford University School of Medicine
San Francisco, Calif.

240. SPIES, T D., STONE, R. E., SUAREZ, R. M., GARCIA LOPEZ, G., LOPEZ-TOCA, R., and REBOREDO A.: *Antianemic properties of reaction products of vitamin B_{12} and the intrinsic factor* J.A.M.A. 151: 1264-1266, April 11, 1953.

Patients with pernicious anemia vary greatly in their requirements for vitamin B_{12}. Some patients respond to small oral doses of vitamin B_{12} without intrinsic factor and some respond only if a source of intrinsic factor is given concomitantly

The authors investigated potent intrinsic factor preparations from several manufacturers and found that these potentiate the effect of vitamin B_{12} in pernicious anemia, nutritional macrocytic anemia and tropical sprue. The effect of one proprietary preparation, tablets of which contain active intrinsic factor and vitamin B_{12}, was studied in 8 patients with pernicious anemia, 1 with nutritional macrocytic anemia and 2 with tropical sprue. These patients had not shown a hematologic response to 10 to 15 mcg. vitamin B_{12} orally (daily?) during a 10 to 30 day control period. The dosage of vitamin B_{12} obtained in this proprietary never exceeded that given during the control period. All 11 patients responded hematologically and clinically

Three patients who were known not to respond to oral vitamin B_{12} even when it was given together with human gastric juice, also failed to respond to this intrinsic factor preparation. They responded well to a single intramuscular injection of 10 mcg of vitamin B_{12}.

A comparative evaluation was made of the responses of 11 cases of pernicious anemia in relapse and 11 cases of tropical sprue in relapse. This was done by finding an amount of intrinsic factor concentrate that, when administered with 10 mcg. of vitamin B_{12}, would produce a definite but submaximal clinical and hemopoietic response in the cases of pernicious anemia. These concentrates were then tested in the patients with tropical sprue. None of the sprue patients had any clinical or hemopoietic response, thus showing that the amount of vitamin B_{12} and intrinsic factor that will produce a definite but submaximal response in the average case of pernicious anemia is inadequate in the average case of tropical sprue.

Northwestern University Medical School, Chicago, Ill.
Hillman Hospital, Birmingham, Ala.
Hospital Mimiya, Santurce, Puerto Rico
General Calixto Garcia Hospital, Havana, Cuba

have no place in treatment. Specific therapy includes measures to supply hemopoietic factors which may be missing (iron, folic acid, vitamin B_{12}, ascorbic acid, dietary amino acids) and to diminish excessive rate of blood destruction.

Microcytic hypochromic anemia is treated with iron-containing foods or iron orally or intravenously

The pathogenesis of macrocytic anemias has been elucidated in recent years. It is now known that both folic acid and vitamin B_{12} are required for normal hemopoiesis and that they participate in widespread metabolic processes. Folic acid is widely distributed in foods, but chiefly in conjugated forms that require enzymatic action for the release of the free vitamin, which is believed to be the only form that can be absorbed. Thus, folic acid deficiency may result from failure of conjugase action. Evidence that the biologically active form of folic acid is *folinic acid has been obtained; ascorbic acid facilitates* the conversion of folic to folinic acid in living tissues. Ascorbic acid plays a part in hemopoiesis both in metabolism of folic acid and in utilization of iron. Appreciable amounts of vitamin B_{12} may become available through bacterial synthesis, but intestinal bacteria that require an exogenous source of this vitamin for growth may utilize it to the deprivation of the host. Intrinsic factor may combine with the extrinsic factor (vitamin B_{12}) forming a microbiologically inactive complex; this may facilitate absorption, perhaps by preventing utilization by coliform organisms. In pernicious anemia the primary factor appears to be lack of intrinsic factor resulting in a conditioned vitamin B_{12} deficiency Dietary lack of folic acid appears to be a more common cause of nutritional macrocytic anemia than lack of B_{12}. Chronic intestinal disorders (idiopathic steatorrhea, disseminated ileitis, partial obstruction, diminished absorptive surface) may impair resorption of both folic acid and B_{12}, resulting in the neurologic changes seen in true pernicious anemia.

Specific measures available for treatment of macrocytic megaloblastic anemias include liver extract, desiccated stomach, vitamin B_{12} and folic acid. Vitamin B_{12} usually constitutes a complete form of replacement therapy for pernicious anemia for macrocytic megaloblastic anemias other than pernicious, folic acid orally (parenterally if intestinal disease is present) or B_{12} orally if pernicious anemia can be excluded, will usually correct dietary deficiency of these vitamins.

In hemolytic anemias the treatment used fo[illegible] caused by defective blood formation is ineff[illegible] cotropin and cortisone have proved of real [illegible] pathic acquired hemolytic anemia they m[illegible] acute crisis, permit reasonably safe spl[illegible] induce long-lasting complete remis[illegible] therapy or need of continuous adm[illegible] hormones.

University of Michigan
Ann Arbor Mich.

239 LUTTGENS, W F: *The ne[illegible] use and abuse,* California Med. [illegible] 1952.

In a review based on 51 published art[illegible] thor discusses the action of vitamin B_{12} fol[illegible] vorum factor and saccharated oxide of iron [illegible]

venously Vitamin B_{12} appears to be the active principle of refined liver extract and is effective in pernicious anemia when given alone parenterally or with a source of intrinsic factor such as normal gastric juice, by mouth. Folic acid usually brings about complete remission in the other types of megaloblastic anemia, and these remissions may be permanent notwithstanding cessation of therapy

Because folic acid can obscure changes in the blood and thus prevent early recognition of pernicious anemia, in turn permitting neurologic disease to develop, inclusion of folic acid in polyvitamin preparations is decried. Saccharated iron is relatively safe for intravenous administration but is of value only in iron-deficiency hypochromic anemia, and is seldom indicated. Overdosage should be avoided because the body cannot excrete iron.

Stanford University School of Medicine
San Francisco, Calif.

240. SPIES, T D., STONE, R. E., SUAREZ, R. M., GARCIA-LOPEZ, G., LOPEZ-TOCA, R., and REBOREDO, A. *Antianemic properties of reaction products of vitamin B_{12} and the intrinsic factor* J.A.M.A. 151 1264-1266, April 11, 1953.

Patients with pernicious anemia vary greatly in their requirements for vitamin B_{12}. Some patients respond to small oral doses of vitamin B_{12} without intrinsic factor and some respond only if a source of intrinsic factor is given concomitantly

The authors investigated potent intrinsic factor preparations from several manufacturers and found that these potentiate the effect of vitamin B_{12} in pernicious anemia, nutritional macrocytic anemia and tropical sprue. The effect of one proprietary preparation, tablets of which contain active intrinsic factor and vitamin B_{12}, was studied in 8 patients with pernicious anemia, 1 with nutritional macrocytic anemia and 2 with tropical sprue. These patients had not shown a hematologic response to 10 to 15 mcg. vitamin B_{12} orally (daily?) during a 10 to 30 day control period. The dosage of vitamin B_{12} obtained in this proprietary never exceeded that given during the control period. All 11 patients responded hematologically and clinically

Three patients [illegible] not to respond to oral vitamin [illegible] together with human [illegible] to this intrin- [illegible] to a single

ANALYTICAL STUDIES

241 JACOBSON B. M., and BISHOP R. C. *Studies of the principle in liver effective in pernicious anemia. III The augmentative effect of accessory factors on the therapeutic activity of vitamin B_{12}*, J Clin. Investigation 28 791 July 1949 (In Soc. Proc.)

The therapeutic action of liver extract in pernicious anemia depends upon the presence of a primary factor which exerts only a slight or moderate hemopoietic effect by itself but is active in the presence of five chemically distinct accessory factors (l-tyrosine, a peptide, xanthopterin, tryptophane, guanosine) These accessory factors are inert without the primary factor Erythrocyte regeneration curves constructed from data in the literature and from personal observation of 16 patients who received a calculated average daily dose of 2 mcg of vitamin B_{12} by parenteral injection were compared with similar curves showing the effect of commercial liver extract in the calculated daily dose of 2.1 U.S.P units The average daily increment of erythrocytes in a range of initial erythrocyte levels from 1.50 to 1.99 millions per cu. mm. was 78 for commercial liver extract, 86 for primary factor with accessory factors, and 59 for vitamin B_{12}. In a range from 2.5 to 2.99 millions per cu. mm., the erythrocyte increments were 63, 61, and 31 respectively The augmentative effect of accessory factors on the action of vitamin B_{12} is illustrated by data on individual cases.

242. LICHTMAN H., WATSON J., GINSBERG V., PIERCE J V., STOKSTAD E. L. R., and JUKES T H.: *Vitamin B_{12}, some properties and its therapeutic use*, Proc. Soc. Exper Biol. & Med. 72 643-645, Dec. 1949

The authors have obtained a pink fraction from concentrated liver extract by chromatography with silicic acid. Upon purification, this fraction yields a red pigment with an absorption spectrum differing from that of vitamin B_{12}. An apparently similar fraction is obtained from cultures of Streptomyces aureofaciens, and crystals prepared from this fraction have been used in the treatment of 5 patients with addisonian pernicious anemia. Intramuscular injection of 1.0, 1.5, or 2.0 mcg. a day in saline solution, gave an optimal hemopoietic response. The reticulocyte peaks obtained by this treatment varied, but in all 5 patients the erythrocyte regeneration was within maximal limits at the end of 21 days of therapy In the single patient who was treated for more than three weeks, the red cell count was nearly normal at the end of seven weeks clinical improvement was also satisfactory

In a sixth patient with addisonian pernicious anemia, the oral administration of 5 mcg. of vitamin B_{12b} for 10 days was ineffective, but when neutralized gastric juice was added, the reticulocytes began to rise slowly on the eighth day

One patient with megaloblastic anemia of pregnancy showed no hematologic response to 1 mcg. of vitamin B_{12b} a day intramuscularly but her severe glossitis cleared completely Later she showed a maximal hematologic response to pteroylglutamic acid.

Kings County Hospital
Brooklyn, N Y
Lederle Laboratories
Pearl River N Y

243 LAJTHA, L. G: *An inhibitory factor in pernicious anaemia serum* Clin. Sc. 9 287-297 1950.

The factor in pernicious anemia serum which inhibits cell maturation is thermostabile and can be overcome in vitro by folic acid or liver extract but not by vitamin B_{12}.

244 KJERULF JENSEN K., and NOER, B.: *Failing effect on pernicious anemia of liver extracts after inactivation of their vitamin B_{12} active material*, Acta pharmacol. et tox. 6 92-96, 1950.

After exposure to the light of an ordinary electric lamp a commercial liver extract lost its characteristic therapeutic effect toward pernicious anemia together with its content of microbiologically active vitamin B_{12} material. (Autoclaving at pH 10 and 121 for 1 hour gave a similar effect.)

The complete failure in efficacy of the liver extract after inactivation of vitamin B_{12} here recorded speaks in favor of the conception of vitamin B_{12} as the predominant substance of liver extracts.

These results indicate that the clinical effect is proportional to the microbiologic B_{12} activity and that a liver extract without vitamin B_{12} content has no clinical effect.

Furthermore the extreme instability of vitamin B_{12} in liver extracts exposed to light indicates that the relationship between clinical response and microbiological activity must be investigated on the same sample or on samples that have been kept under exactly the same conditions.

Blood Cells

245. DEDICHEN J., and LALAND P: *Anti-pernicious-anemia factor and white-cell count*, Lancet 2 282-283, Aug. 13, 1949

In a normal individual, the leukocyte count was uninfluenced by the intramuscular injection of 40 mcg. of pure crystalline anti-pernicious anemia factor but rose significantly following intramuscular administration of 10 cc. of a potent liver extract. One of the authors had previously noted that folic acid in doses of 25 mg. intramuscularly had no effect on the white cell count of normal subjects. These findings indicate that the leukocytosis factor in liver extract reported by Dedichen and fractionated by Laland several years ago is not identical with the pure anti-pernicious anemia factor or with folic acid.

The University Hospital, and
Nyegaard & Co.
Oslo, Norway

246. OCKRENT C. *Relation between vitamin B , and the red blood cells* Nature 165 280-281 Feb. 18 1950 (In Letters to the Editor)

A hypothesis is presented to explain the therapeutic action of vitamin B_{12} in pernicious anemia in what is commonly considered surprisingly small doses. The number of B_{12} molecules in a 10 mcg. dose is calculat
$5x10^{15}$ The average total number of red blood
an adult human in good health is esti
The number of red blood cells may
number or less in a case of pernici

It is apparent that there are more than enough B_{12} molecules for a one-to-one correspondence with the red blood cells and that an excess of B_{12} is available for storage in the liver On B_{12} injection, a rapid rise is observed in the number of reticulocytes, the precursors of the erythrocytes. It therefore appears probable that at least one B_{12} molecule interacts, at an earlier stage of the normoblast process, with one blood cell. It may be that the B_{12} molecule provides in some way an essential unit for each individual blood cell in an early stage of its development.

The British Drug Houses, Ltd.
London, England

247 JONES, O P., and SMITH, A.: *Transmission of antianemic principle across the placenta and its influence on embryonic erythropoiesis. II Comparison of the effect of liver extract and pteroylglutamic acid* (PGA) Blood 5 499-521, June 1950.

Both liver extract and PGA (folic acid) cause a reduction in mean cell diameters, but the former is slightly more effective.

248. LARSEN O: *Vitamin B_{12}*, Lancet 2 407 Sept. 23, 1950 (in Soc. Proc.)

" Dr O Larsen (Norway) described the occurrence in pernicious anaemia blood of large thin cells which he called macrocytes—in contradistinction to megalocytes which are large cells of normal thickness. It is these cells that cause macrocytosis persisting long after the mean cell volume becomes normal under treatment; they have a life-span of 40-60 days, and a considerable proportion of their haemoglobin appears to be foetal. Treatment with vitamin B_{12}, folic acid, and some commercial liver extracts fails to influence the macrocytes, and Dr Larsen suggested that liver contains another factor necessary to produce a completely normal blood picture. This factor had been separated and is not haemopoietic in the usual sense."

249 WATSON C. J: *The erythrocyte coproporphyrin variation in respect to erythrocyte protoporphyrin and reticulocytes in certain of the anemias*, Arch. Int. Med. 86 797-809 Dec. 1950.

In addition to the protoporphyrin previously studied, human erythrocytes contain free coproporphyrin normally not exceeding 2 mcg. per 100 cc. of red blood cells. Studies of the relationship between erythrocyte protoporphyrin, erythrocyte coproporphyrin, and reticulocytes in various anemias, especially in pernicious anemia during the response to vitamin B_{12} therapy suggest that the erythrocyte coproporphyrin value is a rather sensitive chemical index of the rate of hemoglobin synthesis (or attempted synthesis) in the bone marrow

University of Minnesota Hospital
Minneapolis, Minn.

250. MUEHRCKE, R. C., and KARK, R. M. *Negative effects of vitamin B_{12} on blood of healthy individuals, with special reference to eosinophils*, Proc. Soc. Exper Biol. & Med. 77 144-145, May 1951

In patients with pernicious anemia who responded to vitamin B_{12} therapy the authors were surprised to find no increase in eosinophils, such as occurs when liver extract or whole liver is administered to healthy individuals or to patients with pernicious anemia. Studies were made of the blood of 8 healthy subjects (3 adults and 5 children) who were given large amounts of vitamin B_{12} for 28 days. The adults were given 300 mcg. a day orally or 75 mcg. parenterally the children received 10 mcg. a day orally in 4 cases and intramuscularly in the fifth case. The blood of these subjects was not affected. In particular no eosinophilia was observed. Neither allergic nor toxic symptoms were noted during or after therapy The authors conclude that vitamin B_{12} is not the eosinophilic factor present in raw calves liver

University of Illinois College of Medicine, and
Research and Educational Hospitals
University of Illinois
Chicago, Ill.

251 BENARD H., GAJDOS, A., and GAJDOS-TÖRÖK, M.: *Action of vitamin B_{12}, folic acid and liver extracts on the free protoporphyrin content of red blood cells* Nature 167 990-991 June 16, 1951 (Letter to the Editors)

In secondary anemias and in iron deficiency anemia, the free protoporphyrin is increased while in Addison's anemia it is decreased. Experiments with rabbits indicate that agents used in the treatment of pernicious anemia, such as vitamin B_{12}, folic acid, and active liver extracts, produce a considerable increase in the free protoporphyrin content of the erythrocytes. This increase of free protoporphyrin is attributed to the acceleration of the synthesis of the pigment. Rabbits that had been depleted of glycine, which is used in building porphyrin, did not show an increase in free protoporphyrin when injected with vitamin B_{12} or folic acid. When glycine was given again, the normal response to vitamin B_{12} or to folic acid was reestablished.

These investigations demonstrate a new aspect of the mechanism of action of agents effective in the treatment of pernicious anemia. The authors believe that vitamin B_{12}, folic acid, and active liver extracts act, at least in part, in pernicious anemia by restoring the synthesis of protoporphyrin, a synthesis which is defective in this disease.

Clinique Médicale de l'Hotel-Dieu
Paris, France

252. LEVEY S., and ORTEN, J M.: *Vitamin B_{12} and production of polycythemia by cobalt*, J Nutrition 45 487-492, Dec. 1951.

Cobalt does not produce a polycythemia by way of the intermediary formation of vitamin B_{12}.

253 THOMPSON R. B.: *Observations on the effects of vitamin B_{12}, liver extracts, folic acid and thymine on the maturation of megaloblasts in culture*, Blood 7 522-525, May 1952.

Author's summary "Folic acid has been demonstrated to have a direct maturing effect on cultures of megaloblasts obtained from patients suffering from pernicious anemia in relapse. No effect has been observed with either liver extracts, vitamin B_{12} [10 to 100 mcg./cc.] or thymine."

Royal Victoria Infirmary
Newcastle-on-Tyne England

254 HAMILTON H E., SHEETS R. F., and JANNEY C. D: *A hemolytic mechanism in pernicious anemia influenced by B_{12}*, J Clin. Investigation 31 636, June 1952 (in Soc. Proc.)

"Eight patients with pernicious anemia were transfused with fresh blood from normal donors of heterologous, compatible A B-O group and Rh type. The concentrations of donors erythrocytes during periods up to 120 days were measured by serial counts of inagglutinable cells by a modification of Ashby s technique yielding accurate data for mathematical analysis.

"Analysis of the disappearance curves of normal transfused cells showed normal survival with B_{12} started 9 to 12 days before transfusion. In patients beginning treatment 9 days before and 1 4, and 5 days after transfusion, random destruction of the normal erythrocytes, irrespective of age, was demonstrated. In general, the rate of loss increased the later B_{12} was given, with the fastest rate in the untreated patient.

"In six studies the rate of random cell destruction was constant throughout the four month period, despite coincidentally adequate reticulocyte responses and satisfactory rises in total erythrocyte counts. In two studies exhibiting the fastest rate of destruction the rate accelerated about 70 days after transfusion.

"Conclusions: (1) Fresh normal erythrocytes are so damaged in a few days in the milieu of the human body deficient in B_{12} that they are destroyed in random fashion, presumably by the trauma of circulation. (2) The cell damage incurred in the first few days is not reversible by the subsequent administration of B_{12}. (3) When severe grades of erythrocyte damage have occurred, the chemical changes of aging or the trauma of 70 days of circulation further hasten destruction. (4) These studies indicate that 45 micrograms of B_{12} weekly must be present in the body for at least 10 days before a safe environment is provided for normal erythrocytes. Until further evidence is available, it is assumed that the hemolytic mechanism exerts similar effects on the circulating erythrocytes of the patient."

Iowa City Iowa

255. DEGOWIN, E. L., HAMILTON H. E., SHEETS R. F., JANNEY C. D., and ELLIS J A. *The role of maturation arrest in the low erythrocyte counts of pernicious anemia*, J Lab. & Clin. Med. 40 790-791, Nov 1952 (in Soc. Proc.)

"Fresh normal erythrocytes belonging to group O were transfused to six patients with pernicious anemia in relapse belonging to group A or B Donors cells were identified in the circulation of the patients by Ashby s method of differential agglutination. Analysis of inagglutinable erythrocyte counts demonstrated that the normal donors' erythrocytes were so affected by twenty four hours or more in the contact with a deficiency of B_{12} in the body of the patients that the transfused cells were randomly destroyed at rates that could be measured rather accurately

"At the beginning of the transfusion experiments the erythrocyte levels of the patients were in steady states with counts of 1.0, 1.64, 1 70, 2.00, 2.00, and 3.5 millions per cubic millimeter In each the aspirated bone marrow presented the cytologic picture which has been considered typical of 'maturation arrest. The subsequent administration of B_{12} parenterally resulted in prompt rises of the patients erythrocyte counts to normal and disappearance of all symptoms and signs of the disease except those attributed to permanent changes in the spinal cord.

"Assuming a normal rate of erythropoiesis when the cells are being destroyed by a hemolytic mechanism, the resulting lowered level of the erythrocyte count can be calculated, if the rate of destruction is known. In these studies it was assumed that the rates of destruction of the patient s erythrocytes were similar to the measured rates at which the biologically tagged cells disappeared.

"In four patients the observed levels of erythrocyte counts before treatment coincided with those calculated on the assumption of normal erythropoietic rates and the observed random loss of donors cells. The lowered cell counts could therefore be explained completely by the action of a hemolytic mechanism and without maturation arrest of significant magnitude. In the other two patients this method of calculation suggested that about one-half of the lowering of the erythrocyte count could be attributed to a hemolytic mechanism, whereas the other one-half was explainable by a phenomenon such as maturation arrest. Reticulocyte response to B_{12} was significantly lower than expected in those in which the hemolytic mechanism predominated."

256 JANNES, J: *Studies on the vitamin B_{12} content of human blood plasma*, Ann. med. exper et biol. Fenniae 30 Fasc. 3-4 329- 1952 (abstr J Am. Dietet. A. 29 158, 160, Feb. 1953)

"Results are given of studies of the vitamin B_{12} level of the blood plasma of five healthy persons and of two patients with pernicious anemia. The method by which the determinations were made is described."

Bone Marrow

257 SIGNY A. G: *Recent work on vitamin B_{12}*, Lancet 1 354-355, Feb. 25, 1950 (in Soc. Proc.)

At a meeting of the section of experimental medicine of the Royal Society of Medicine, Dr Signy "reported what he thought was an effect on the bone-marrow one hour after injection [of B_{12}] in a patient in whom the marrow was studied hourly"

258. MOLLIN D L., and DACIE, J V: *Recent work on vitamin B_{12}. Observations on the relationship between the red cell and reticulocyte responses and changes in the bone-marrow of patients suffering from pernicious anaemia treated with injections of liver extracts or vitamin B_{12}*, Proc. Roy Soc. Med. 43 541-546, July 1950

259 DACIE, J V: *Recent work on vitamin B_{12}*, Lancet 1: 354, Feb. 25, 1950 (in Soc. Proc.); Proc. Roy Soc. Med. 43 541-546, July 1950.

The author has described "30 cases of true pernicious anaemia treated with liver extract, vitamin B Lester Smith s unnamed substance. It was a

finer relations between the histological picture of the bone-marrow the size of the reticulocyte response, and the increase in the red cells. With a single dose of 20 μg. of vitamin B_{12} the marrow became normoblastic in the first five days, and reticulocytes might account for nearly all the red-cell increase. In some cases the marrow reverted to an intermediate stage as early as the 5th day and was megaloblastic by the 10th day Dr Dacie found that the red-cell response was biphasic, with a shelf between the 5th and the 9th day During the second rise there were fewer reticulocytes, and almost all the increase was due to cells already mature as they reached the blood-stream. The size of the second rise depended on the dose and could be correlated with the marrow picture. A single initial dose of 30-40 μg. caused the marrow to remain normoblastic to the 15th day; 20 μg. gave a satisfactory response in the blood but allowed a regression of the marrow to the megaloblastic stage in the same period."

260. MAGNUSSEN J D: *L'influence de l'extrait de foie et de la vitamine B_{12} sur la production des érythrocytes in vitro (The influence of liver extract and vitamin B_{12} on the production of erythrocytes in vitro)* Acta pharmacol. et toxicol. 6 263-268, 1950 (abstr Semaine d. hôp. de Paris 27 107 Jan. 6, 1951)

Vitamin B_{12} can completely replace liver extract as a nutritive element for the cellules of the marrow in the study of the development of erythrocytes in vitro. It is probable that the effect of the liver extract is due to its vitamin B_{12} content. Probably vitamin B_{12} does not have the same action in vivo, for the concentrations used in vitro exceed those that can be obtained in the blood. These results were obtained partly by spectrographic study and partly by study of the growth of Lactobacillus Dorner

261 FADEM, R. S., and BERLIN, I *Comparisons between bone marrow differentials prepared from particles and from random samples of aspirate and determinations of the dilution of aspirate with peripheral blood utilizing radioactive phosphorus (P^{32})* Blood 6 160-174, Feb. 1951.

In an article in which bone marrow differentials are compared, a case of pernicious anemia in relapse is described. The patient was successfully treated with parenteral vitamin B_{12} after oral vitamin B_{12} had been ineffective.

Veterans Administration Hospital
Bronx, N Y

262. EDITORIAL: *Vitamin B in the bone marrow* South. M J 44 361-362, April 1951.

The results obtained by Horrigan, Jarrold, and Vilter who studied the effects of folic acid and vitamin B_{12} when instilled into the marrow of patients with pernicious anemia in relapse (*J Clin. Investigation* 30 31, Jan. 1951) are summarized. Folic acid was ineffective when administered thus; it probably requires alteration inside the body before it can stimulate the bone marrow B_{12}, unaltered by stomach or liver corrects a qualitative abnormality in cellular ribonucleic acid of patients with pernicious anemia in relapse. Both folic acid and B_{12} are believed to be concerned in the formation of nucleic acid. In patients with pernicious anemia in relapse, ribonucleic acid is thought to be abnormally distributed, and this is corrected by vitamin B_{12}. Both folic acid and B_{12} correct abnormal erythrocyte maturation, the first only after a structural change in the body the second by direct reaction with the marrow components after injection into the marrow cavity

263 ETESS, A. D., and LITWINS J: *Pernicious anemia with early marrow change following therapy* New York State J Med. 51 2787-2788, Dec. 1 1951.

After the diagnosis of pernicious anemia had been made in a patient, a sternal marrow needle was inserted and marrow was aspirated. Following the administration of 60 mcg. of vitamin B_{12} intramuscularly marrow specimens were withdrawn every 15 minutes for three hours, and daily for three days. Within two and a half hours there was a decrease in megaloblasts (from 15 to 1.8%) and a corresponding increase in normoblasts (from 49 to 74%) Peripheral smears showed no change in the size of the erythrocytes, and the reticulocytes did not increase. It is pointed out that after the administration of vitamin B_{12}, a diagnosis of pernicious anemia cannot be made with certainty Liver extract or vitamin B_{12} should not be given until a diagnosis is made, to prevent the masking of the signs of the disease.

Beth Israel Hospital
New York, N Y

264 STRAUSS, M. B., BROKAW R., and CHAPMAN C. B.: *Leukemoid bone marrow in pernicious anemia*, Am. J M. Sc. 223 54-60, Jan. 1952

Authors summary and conclusions "Two patients with Addisonian pernicious anemia complicated by infection presented bone marrow pictures characterized by intense granulocytic activity and almost devoid of megaloblasts. In each the diagnosis of aleukemic myelogenous leukemia was considered. Both responded uneventfully to vitamin B_{12} therapy and subsequently exhibited entirely normal marrows. It is concluded that the bone marrow in pernicious anemia in relapse is not always pathognomonic."

In each case the first dose of vitamin B_{12} was 45 mcg. One patient received a maintenance dose of 15 mcg. every week, and the other received 45 mcg. every week.

Cushing VA Hospital
Framingham, Mass.
Veterans Administration Hospital
Minneapolis, Minn.

CELL TYPES, CONTENTS

265. HORRIGAN D., and VILTER, R. W: *Direct action of vitamin B_{12} upon human bone marrow the effect of instillations of vitamin B_{12} and folic acid into the bone marrow as studied by histochemical techniques* Program 42nd Annual Meeting, Am. Soc. Clin. Investigation, May 1, 1950, p. 33 (advance abstr); J Lab. & Clin. Med. 29 823, June 1950 (in Soc. Proc.)

In 5 patients with megaloblastic anemia, 1 mcg. of vitamin B_{12} or 1 mg of folic acid was injected into one

iliac crest marrow cavity and 40 hours later marrow was aspirated from the same site and also from the opposite iliac crest. From the findings the authors conclude that vitamin B_{12} can be utilized locally by bone marrow cells and it corrects a qualitative abnormality in cellular ribonucleic acid of persons with pernicious anemia folic acid, however cannot be utilized locally and probably must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body

Cincinnati, Ohio

266. HORRIGAN D., JARROLD T., and VILTER, R. W *Direct action of vitamin B_{12} upon human bone marrow the effect of instillations of vitamin B_{12} and folic acid to the bone marrow as studied by nucleic acid staining techniques*, J Clin Investigation 30 31-36, Jan. 1951

The action of vitamin B_{12} and of folic acid upon human bone marrow was studied in 6 persons with pernicious anemia in relapse. Three received injections of 1 cc. of solution containing 1 mcg. of vitamin B_{12} and 3 received injections of 1 cc. of solution containing 1 or 2 mg. of folic acid. In each case the injection was made into the marrow cavities of the iliac crests after aspiration of 2 to 3 cc. of marrow and blood. Forty-eight hours later bone marrow was aspirated from the exact site of the previous instillation and also from the opposite iliac crest. The aspirated specimens were stained to permit differential cell counts and demonstration of cytoplasmic ribonucleic and desoxyribonucleic acids.

It was found that vitamin B_{12} can be utilized locally by the bone marrow cells and corrects a qualitative abnormality in cellular ribonucleic acid in persons with pernicious anemia in relapse. It need not be altered by stomach or liver to exert this effect. Folic acid is not utilized locally by bone marrow within 48 hours after instillation into the marrow cavity but when given orally or parenterally it has the same cytologic and cytochemical effects as vitamin B_{12}. It is probable that folic acid must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body

University of Cincinnati College of Medicine
Cincinnati, Ohio

267 HORRIGAN D L., and HEINLE, R. W *Role of tissue binding in local erythroid maturation following instillation of vitamin B_{12} into marrow cavity* J Clin. Investigation 31 639 June 1952 (in Soc. Proc.)

"A local erythroid maturation effect following instillation of vitamin B_{12} directly into marrow cavity of persons with pernicious anemia was described previously The interpretation of this observation was that the vitamin was capable of inducing maturation without first being altered.

"Other investigators have shown that vitamin B_{12} exists in the blood in bound form that many substances are capable of binding, and that the unbound vitamin is ineffective in causing erythroid maturation in vitro.

"In this study the observations indicate that the vitamin is bound at the site of instillation but that binding capacity is limited. After instillation of 1 mcg. into the marrow cavity of three patients, previously reported and of 0.25 mcg in another maturation occurred only at the site of injection without other hematologic response. In two patients, however instillation of 1 mcg. was followed by a systemic response, and generalized marrow maturation, although the latter was more advanced at the site of instillation.

"These findings suggest that in the patients who showed local maturation only the vitamin was completely bound at the injection site, whereas in those having a systemic response, only part was bound locally the remainder being available for systemic utilization. Local binding capacity is thought to be related to the degree of tissue vitamin deficiency and varies in different patients. That the effective binding substance and gastric intrinsic factor are the same has been suggested but not proven. If combination with a binding substance is necessary before vitamin B_{12} becomes active, a deficiency of tissue binding substance might be the basic defect in certain refractory macrocytic anemias associated with megaloblastic or abnormal normoblastic bone marrow states as have been described in cirrhosis and certain other conditions."

Cleveland, Ohio

268. REISNER, E. H., Jr., and KORSON R.: *Microspectrophotometric determination of desoxyribosenucleic acid in megaloblasts of pernicious anemia*, Blood 6: 844-849 April 1951.

Authors summary "In 9 patients with various types of megaloblastic anemia responding to treatment with vitamin B_{12}, folic acid or liver extract, no significant deviations from the normal amounts of total or polymerized DNA were observed in the nuclei of red blood cells in marrow smears.

"During the maturation of megaloblasts in the bone marrow there is a gradual loss of nuclear DNA.

"This pattern is quantitatively and qualitatively similar for normal marrow and for that of pernicious anemia in relapse and after treatment."

New York University Post Graduate Medical School,
Bellevue Hospital, and
Columbia University
New York, N Y

269 DAVIDSON J N., LESLIE, I., and WHITE, J C.: *The nucleic-acid content of the cell*, Lancet 1 1287 1290, June 16 1951.

In experiments on the desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of cells, the authors studied the changes in these substances in normal cells and during the course of various hematologic conditions. These acids were determined as the phosphorus compounds (D.N.A.P and R.N.A.P)

One of the conditions studied was megaloblastic anemia. These anemias had the greatest and most consistent deviations from normal in the amounts of cellular nucleic acids. Individual variations were generally greater with more severe anemia. In 15 observations on 8 megaloblastic anemia patients treated with liver or vitamin B_{12}, the mean amount of D.N.A.P per cell did not change significantly during the hemopoietic response. T

amount of R.N.A.P declined rapidly toward normal, although the mean remained significantly high. The authors state "The decline in amount of R.N.A.P was strikingly related to the return of the marrow to normoblastic erythropoiesis and to the improvement in the peripheral blood picture."

The authors comment that one advantage of knowing the D.N.A. content of the nucleus is that a determination of D.N.A. as well as other chemical constituents in a tissue sample makes possible calculation of the amount of such constituents in each cell. It is thus possible to express the composition of a tissue in terms of the average composition per cell. This method is of particular interest in considering megaloblastic anemia, in which the D.N.A. content of the nucleus is significantly high, and the greatly increased R.N.A. content of the cell explains the pronounced basophilia of the cytoplasm of younger megaloblasts.

University of Glasgow
Glasgow, Scotland
Postgraduate Medical School of London
London, England

270. MENTEN M. L., and WILLMS M.: *Nucleic acid in cells of bone marrow of patients with pernicious anemia changes in content before and after specific treatment*, A.M.A. Arch. Path. 54 351-358, Oct. 1952.

The effect of specific therapy of pernicious anemia with vitamin B_{12} or liver extract on the nucleic acid content of bone marrow cells was studied in 11 patients. Bone marrow aspirations were made before and after therapy was begun. The results are described as follows: "The desoxyribonucleic acid phosphorus content per cell of bone marrow aspirated from patients with pernicious anemia ranged from 1.80 to 0.50 mg. x 10^{-9} The ribonucleic acid phosphorus varied from 1.24 to 0.30 mg. x 10^{-9} The higher values were obtained in patients with anemia and symptoms of moderate severity the lower values were found in patients in relapse or with inadequate treatment over a variable time. Specific treatment caused in the bone marrow a rapid disappearance of megaloblasts with an increase in polychromatic erythroblasts and a decline in cellular nucleic acid lasting from 3 to 30 days. The duration of the decline was proportional to the degree of reduction in the initial values of cellular nucleic acid, percentile rise in polychromatic erythroblasts, and the height of the response of reticulocytes." The addition of folic acid or ascorbic acid in 2 of the patients to the B_{12} regimen did not hasten or improve the reparative effects of vitamin B_{12}.

British Columbia Medical Research Institute, and
Vancouver General Hospital
Vancouver B. C., Canada

Metabolic Processes

271. KREVANS, J R., CONLEY C. L., and BARROWS, C. R. *Observations on the absorption and excretion of vitamin B_{12}*. Bull. Johns Hopkins Hosp. 88 568-569 June 1951 (in Soc. Proc.)

"Crystalline vitamin B_{12} was given parenterally to normal individuals and to patients with treated and untreated pernicious anemia. Urine collected following injection was assayed microbiologically for B_{12} activity Following the intramuscular injection of 25 micrograms or less of vitamin B_{12} there was no measurable increase in the B_{12} activity of the urine. When 100 micrograms were injected intramuscularly the B_{12} activity of the urine collected in the first 8 hours accounted for approximately 40 per cent of the injected vitamin. There was little additional excretion of vitamin B_{12} after the first 8 hours. When 500 micrograms or more of B_{12} was given parenterally approximately 100 per cent of the injected vitamin could be accounted for in the urine in the first 8 hours. There was no measurable difference in the urinary excretion of vitamin B_{12} of normal individuals and of patients with pernicious anemia. The administration of single oral doses of vitamin B_{12} in amounts as large as 10,000 micrograms was not followed by a measurable increase in the B_{12} activity of the urine in normal individuals or in patients with pernicious anemia. Nevertheless 4 patients with pernicious anemia in relapse showed striking hematologic responses when given single oral amounts of vitamin B_{12}. Patients receiving 3000 or more micrograms of B_{12} had complete hematologic remissions without further therapy The absorption and excretion of vitamin B_{12} was studied further using B_{12} containing radioactive Cobalt 60. Five micrograms of the labelled vitamin were administered orally to a normal subject. Feces excreted in the following 4 days was homogenized, ashed, extracted with a-nitroso-β-naphthol, and electroplated on copper discs from a saturated solution of ammonium oxalate. The radioactive Cobalt recovered accounted for 4.8 of the 5 micrograms administered. These observations indicate that vitamin B_{12} is poorly absorbed from the normal gastro-intestinal tract."

The authors had expected to find that patients with pernicious anemia would not absorb orally administered vitamin B_{12} and therefore would not excrete it in the urine. This, however was not the case. Had it been so, determining the urinary excretion of orally administered vitamin B_{12} might have been usable as a diagnostic test in patients "with pernicious anemia who had no anemia at the time of examination." The authors plan to administer radioactive vitamin B_{12} intravenously to patients in the near future.

Johns Hopkins Hospital
Baltimore, Md

272. CONLEY, C. L., KREVANS J R., CHOW B. F., BARROWS C., and LANG, C. A. *Observations on the absorption, utilization, and excretion of vitamin B_{12}*. J Lab. & Clin. Med. 38 84-94, July 1951.

Authors summary "Parenteral administration of as little as 25 micrograms of vitamin B_{12} to patients with pernicious anemia in severe relapse may be followed by complete hematologic remission. When vitamin B_{12} is administered orally in a single dose without gastric juice, approximately 5,000 micrograms are required to produce the same effect.

"Following parenteral administration of vitamin B_{12} in amounts larger than 25 micrograms, there was prompt urinary excretion of the vitamin. When 500 micrograms or more were injected, the urinary excretion of B_{12} was approximately quantitative. No difference was detected between the B_{12} excretion of normal individuals and of patients with pernicious anemia.

"Oral administration of single amounts of vitamin B_{12} as large as 10,000 micrograms was not followed by a measurable increase in the B_{12} content of the urine of normal individuals or of patients with pernicious anemia."

The Johns Hopkins University and Hospital
Baltimore, Md.

273. MOLLIN D L., and ROSS G I M.: *The vitamin B_{12} concentrations of serum and urine of normals and of patients with megaloblastic anaemias and other disease*, J Clin. Path. 5 129-139 1952 (abstr Am. J Clin. Path. 23 382, April 1953)

"Vitamin B_{12} microbiologically determined, was found to be present in combined and free forms. Total values of 100 to 720 µµg. (mean 358 µµg.) per ml. of serum were found in normal subjects, and combined values of 100 to 620 µµg. (mean 329 µµg.) Patients with megaloblastic anemia in the main showed lower than normal values, but patients with other diseases showed normal B_{12} values."

274. HAUSMANN K., and MULLI, K. *Study of metabolism of folic acid and of citrovorum factor* Acta haematol. 7 1 Jan. 1952 (abstr J.A.M.A. 149 90, May 3, 1952)

"Citrovorum factor was given a therapeutic trial in 10 patients with pernicious anemia, 2 with symptomatic megaloblastic anemia, 3 with agranulocytosis, and 2 with idiopathic aplastic anemia. Two patients with pernicious anemia were given 6 mg. of synthetic citrovorum factor daily by mouth, but did not respond, and one who was given 9 mg. daily by mouth obtained only a suboptimal hematopoietic effect. In nine patients who were given 5 to 15 mg. of synthetic citrovorum factor daily intramuscularly the erythrocyte level was restored to nearly normal and the megaloblasts in the bone marrow disappeared. One patient was given a single massive intramuscular injection of 40 mg. of citrovorum factor obtained from bovine liver without significant effect on the hematopoiesis. One of the two patients with symptomatic megaloblastic anemia did not respond to the synthetic citrovorum factor while the number of erythrocytes was restored nearly to normal in the other The patients with aplastic anemia and agranulocytosis did not respond to the treatment. Microbiological assays of the urine following administration of folic acid and citrovorum factor directly into the colon showed little evidence of absorption from this organ, but following oral or parenteral administration the urinary output increased significantly Folic acid in high doses was found to inhibit the growth-promoting effect of vitamin B_{12} on L. Leichmannii; high doses of citrovorum factor thymine and thymidine did not exert such an antagonistic effect. Studies of the gastric juice of patients with pernicious anemia revealed reduced catalase and increased peroxidase activity by an oxidative process the citrovorum factor is transformed to folic acid or to a substance similar to folic acid, while vitamin B_{12} and vitamin C are destroyed. These results may explain the pathogenesis of neurological symptoms and the favorable effect of antibiotics on pernicious anemia."

275 GIRDWOOD R. H. *Some aspects of the metabolism of anti-megaloblastic substances in man*, Blood 8: 469-485, May 1953.

Author's summary "1 When the sera of pernicious anemia patients or controls were heated at 100 C. for 30 minutes, they developed the ability to support the growth of L. leichmannii by virtue of some substance other than vitamin B_{12}. It seemed likely however that following the administration of the vitamin, such heating also liberated free B_{12} in the serum from a combined form.

"2. The L. leichmannii assay did not appear to be satisfactory for showing possible difference in the levels of vitamin B_{12} in the urines or sera of pernicious anemia patients and controls.

"3. Parenterally administered vitamin B_{12} did not cause any measurable rise in the serum level of folic acid or citrovorum factor in pernicious anemia patients or controls.

"4. The synthetic folic acid conjugates pteroyldiglutamic acid and pteroyltriglutamic acid appeared in the sera and urines of pernicious anemia patients as pteroyl glutamic acid or some related substance with folic acid activity for S. faecalis.

"5. Orally administered citrovorum factor appeared to be largely converted by the gastric juice to folic acid if free hydrochloric acid was present. When administered parenterally however citrovorum factor was excreted in the urine largely unchanged."

University of Edinburgh,
Edinburgh, Scotland

276. UNGLAUB, W G., ROSENTHAL, H. L., and GOLDSMITH, G A.: *Vitamin B_{12} in blood and urine after oral and parenteral administration*, Federation Proc. 12 432, March 1953.

"Following intramuscular administration of 10 to 100 µg. of vitamin B_{12} to normal subjects, serum vitamin B_{12} activity rose in one hour to maximum values which were proportional to the amounts injected. Urinary vitamin B_{12} activity in 24 hr following injection increases with the quantity administered, but cannot be predicted accurately Serum vitamin B_{12} activity during fasting is consistently lower in patients with untreated macrocytic anemia than in normal subjects. In 2 patients with pernicious anemia in relapse who received 25 µg. of vitamin B_{12} intramuscularly increases in serum activity did not differ from those of normal subjects but vitamin B_{12} activity in urine was somewhat less than normal. Serum vitamin B_{12} activity did not increase significantly when normal subjects were given 500 and 1000 µg. orally After oral administration of 3000 µg., serum activity reached maximum values equal to those found after intramuscular injection of 10-25 µg. Serum activity remained elevated for longer periods after oral than after intramuscular administration. In 3 of 4 patients with macrocytic anemia who received 3000 µg. of vitamin B_{12}, orally serum activity increased to maximum value similar to those noted after intramuscular injection of 1-50 µg. In the 4th no significant rise occurred. In a 5th patient serum activity increased after oral administration of 500 and 1000 µg. Optimal hematologic response resulted in all patients. Following oral doses, no significant increase in vitamin B_{12} activity was found in the urine."

Tulane University School of Medicine
New Orleans, La.

277 MEYER, L. M. *Oral administration of Co^{60} vitamin B_{12} in pernicious anemia*, Proc. Soc. Exper Biol. & Med. 82 490-491, March 1953.

Author's conclusions "Following oral administration of 1.0 µg. of Co^{60} vit. B_{12} 1. Seven normal persons excreted 12-59% of radioactive cobalt in the stool in 3-9 days. 2. No activity could be found in the urine of 2 normal subjects up to 8 days after ingestion of the vitamin. 3. Four patients with pernicious anemia in remission excreted 89-99% of the dose in the stool. 4. In 3 of these cases 2 mg. of folic acid, 10 mg. of folic acid for one week, 50 g. of ventriculin, and 15 mg. of intrinsic factor had no appreciable effect on the amount excreted. 5. In one case 2 mg. of folic acid and 15 mg. of intrinsic factor reduced the excretion of Co^{60} about 40% and 50 g. of ventriculin cut the fecal activity about 25% 6. In 3 patients 2 g. of aureomycin for one week had no effect and 150 cc. of neutralized normal gastric juice reduced the fecal activity about 30%"

Goldwater Memorial Hospital
New York, N Y
Swedish Hospital
Brooklyn, N Y

DIURESIS

278. BARNARD R. D., and WEITZNER, H. A. *B_{12} diuresis*, Lancet 2 717 Oct. 15, 1949 (In Letters to the Editor)

Diuresis has been noted to attend the administration of liver extract or crystalline vitamin B_{12} to patients with addisonian anemia. That this might be explained by a direct diuretic effect of B_{12} was suggested by the prompt disappearance of edema following injection of 15 mcg. of the vitamin in an amyotrophic patient who had developed water retention while on adrenal cortex therapy. To test the point, 16 patients with essentially normal blood pictures were given 45 to 75 mcg. of vitamin B_{12} in a single injection. During the ensuing 24 hours, 12 responded with an unequivocal increase in urinary output. These 12 included 1 patient with amyotrophic lateral sclerosis, 3 patients with chronic glomerulonephritis, 4 with addisonian anemia in remission, 1 patient with cardiac decompensation, and 3 normal volunteers. The 4 subjects showing no definite urinary response included 2 volunteers and 2 patients with cardiac decompensation. Le Goff's earlier observations on the diuretic effect of cobalt seem to offer a more satisfactory explanation for the diuresis following B_{12} administration than the usual explanation offered, that is, changes in hematomorphology. The possible clinical utility of vitamin B_{12} as a diuretic is suggested.

New York, N Y

279 JALILI, M. A. *Vitamin B_{12} diuresis*, Lancet 1 977 May 20, 1950 (In Letters to the Editor)

The writer reports 4 cases treated with single injections of 40 to 60 mcg. of vitamin B_{12}. In no case was any diuresis observed. This is contrary to the findings of Barnard and Weitzner (Lancet 2: 717 1949 [Abstr 278]) who observed diuresis in 12 of 16 subjects after single injections of 45 to 75 mcg. of vitamin B_{12}.

Royal Faculty of Medicine
Baghdad, Iraq

280. BEDFORD P D *Diuretic effect of vitamin B_{12}*, Lancet 1 1232-1233, June 2, 1951 (In Letters to the Editor)

The author describes his experiment for determining the diuretic effect of vitamin B_{12} in 37 patients with normal blood pictures. Vitamin B_{12} was given in single intramuscular 100 mcg. doses to patients on diets containing not more than 650 mg. of sodium per day and permitting free intake of fluids. In no case was there a significant diuresis (defined as output exceeding intake) after vitamin B_{12}. Mercurial diuretics caused diuresis in each case although occasionally this was within the normal range for the patient.

Cowley Road Hospital
Oxford, England

EXCRETION OF VITAMIN B_{12}

281. CONLEY C. L., LANG C. A., CHOW B. F., and ELLICOTT C. E.: *B_{12} activity of the urine of normal subjects and of patients with pernicious anemia following oral and parenteral administration of the vitamin*, J Clin. Investigation 29 806 June 1950.

282. CHOW B. F., LANG, C. A., DAVIS, R., CONLEY C. L., and ELLICOTT C. E.: *The appearance of vitamin B_{12} activity in urine after oral and intramuscular administration to man*, Bull. Johns Hopkins Hosp. 87 156-163, Aug. 1950

Vitamin B_{12} was given intramuscularly and orally to normal subjects and to untreated patients with pernicious anemia, after which the vitamin B_{12} activity of the urine was determined by microbiological assay

Two normal subjects were given 100 mcg. of crystalline vitamin B_{12} intramuscularly Urine collected prior to the injections showed no appreciable B_{12} activity However in the first 12 hours following the injection, the B_{12} activity of the urine was equivalent to the activity of about 50 per cent of the injected material. Three patients with untreated pernicious anemia were given 100 mcg. of crystalline vitamin B_{12} intramuscularly Most of the vitamin administered appeared in the urine.

In two normal subjects given 100 mcg. of crystalline vitamin B_{12} orally urine collected over a subsequent 48-hour period showed no detectable B_{12} activity

Three normal subjects were given a larger dose (500 mcg.) of vitamin B_{12} intramuscularly and 3 others received 500 mcg. of the vitamin orally In the 3 subjects receiving the oral dose, the vitamin did not appear in the urine. However B_{12} activity promptly appeared in the urine of the 3 subjects receiving the vitamin parenterally The B_{12} activity of the urine diminished rapidly about eight hours after the injection.

The failure of B_{12} activity to appear in the urine of normal subjects after oral administration of the vitamin may indicate that it is poorly absorbed from the gastrointestinal tract even by normal individuals. The authors had anticipated that vitamin B_{12} activity would appear in the urine of normal subjects after oral administration but that the activity would not appear when the vitamin was given orally to patients with pernicious anemia.

It is conceivable that vitamin B_{12} is altered in some way during its passage through the gastrointestinal tract so that it is no longer capable of being excreted by the kidneys, or that it might undergo some change during its absorption which would render it ineffective in meeting the nutritive requirements of the microorganism employed in the assay

The concomitant administration, by either the oral or parenteral route of biotin and vitamin B_{12} was followed by the appearance of free biotin in the urine.

Johns Hopkins University
Baltimore, Md.

283. SOKOLOFF M. F., SANNEMAN E. H., JR., and BEARD M. F *Urinary excretion of vitamin B_{12}*. Blood 7 243-250, Feb. 1952.

The urinary excretion of vitamin B_{12} was studied in 12 patients, 6 of whom had pernicious anemia. Dosages used were 42.2, 63.3, 84 4 211.0 mcg. given parenterally both in ascending and descending order at daily intervals. The two larger amounts resulted invariably in the excretion of 53 to 68 per cent of the injected vitamin B_{12} within 18 hours. The percentage of excretion rose remarkably as the dosage increased. When 42.2 mcg. was given, there was relatively little excretion when this was the initial dose, but the excretion was appreciable when these doses followed the injection of the larger doses. "Saturation" of the body apparently occurs. In general more vitamin B_{12} is excreted in the smaller doses if these follow the larger doses. No significant differences could be detected between the pernicious anemia and nonpernicious anemia patients.

University of Louisville School of Medicine
Louisville, Ky.

284 CUTHBERTSON, W F J: *Clinical and laboratory aspects of vitamin B_{12}*. Lancet 2 133-134, July 19 1952 (in Soc. Proc.) *also* Brit. M. J 2 153-154 July 19 1952 (in Soc. Proc.)

In a study of the excretion of vitamin B_{12}, it was found that maximal urinary output occurred some four hours after intramuscular injection of 200 mcg. little was excreted after seven hours. With injection of 1,000 mcg., 50 per cent of the dose was eliminated by a normal person and about 60 per cent by patients with pernicious anemia. Less of the vitamin was excreted when the anemia was in remission than during relapse. Following doses of 3,000 to 9,000 mcg. orally excretion was much less than expected. Possibly the vitamin was taken up by the intestinal mucosa into the abdominal lymphatics, or in the liver "Lymphatic absorption seemed unlikely because the excretion following intramesenteric injections at laparotomy was much the same as that after intramuscular injection."

285 ROSS G. I M. *Clinical and laboratory aspects of vitamin B_{12}*, Brit. M. J 2 154, July 19 1952 (in Soc. Proc.) Lancet 2 134, July 19 1952 (in Soc. Proc.)

"Dr G I M. Ross (London) reported on assays depending on the use of *Euglena gracilis* which was sensitive to 1 μμg./ml. In patients with megaloblastic anemia due to lack of vitamin B_{12} the level of B_{12} in the serum was nearly always low while if the megaloblastosis was due to folic acid lack, the B_{12} levels were normal. In about 6% of B_{12} deficient subjects a single estimate might give falsely high values, but repeated tests gave lower figures. When injected, vitamin B_{12} combined with serum protein in the course of eight hours, and during this period some of the uncombined B_{12} was excreted thereafter a constant level was reached which persisted for some days. The concentration of B_{12} in the serum and the duration of the rise depended mainly on the dose administered but also on the subject. Saturation occurred after injections given daily for 7 to 10 days."

286. MONTO R. W: *Respiratory tract absorption of crystalline B_{12} in man demonstrated by urinary bioassay and hematopoietic studies* Federation Proc. 12 396, March 1953.

"After oral administration of B_{12} at dosages as high as 10,000 mcgm., B_{12} growth activity in the urine has been shown to be less than 0.05 millimicrograms/ml. Upon *inhalation* of 200 mcgm. of B_{12} in saline, B_{12} activity in the urine reached the level of 0.26 millimicrograms/ml. and following inhalation of approximately 500 mcgm. of B_{12} in lactose powder B_{12} activity in the urine rose to 0.134 millimicrograms/ml. Direct pulmonary instillation of 100 μg. of crystalline vitamin B_{12} by means of the bronchoscope, resulted in a urinary excretion (9 76 millimicrograms/ml.) pattern equivalent to the intramuscular injection of 60-80 mcgm. of B_{12}. Inhalation therapy with crystalline B_{12} in physiological saline and lactose dust has resulted in adequate clinical and hematological response in five patients with pernicious anemia in relapse. A sixth patient demonstrated maximal response to a single intranasal application of nose drops of 100 μgm. crystalline B_{12} in 1 ml. of saline. Twenty five pernicious anemia patients in remission have been satisfactorily maintained by inhalation treatment for periods up to 1 year"

Henry Ford Hospital
Detroit, Mich.

CHEMISTRY

287 ABBOTT L. D., JR., and JAMES G W., III: *Effect of vitamin B_{12} on the urinary phenol fractions in pernicious anemia*, J Lab. & Clin. Med. 35 35-42, Jan. 1950.

Authors summary: "The effect of crystalline vitamin B_{12} on the excretion of tyrosine metabolites in pernicious anemia was studied by fractionating the ether soluble urinary phenols into a fraction containing the hydroxyphenyl acids (A) and a fraction containing ether soluble phenols not soluble in sodium bicarbonate (B) Three of the four patients studied were noted to have elevated pretreatment phenol fraction ratios (A:B) which decreased after the parenteral administration of crystalline vitamin B_{12}. In two of these patients the decrease in ratios was noted to precede the reticulocyte response. The one patient who did not have elevated pretreatment ratios was a Negro man who responded to vitamin B_{12} therapy with an increase in ratios which preceded reticulocytosis. Crystalline vitamin B_{12} in the very small amounts needed to produce remission in pernicious anemia was found to have a prompt effect on tyrosine metabolism as indicated by these variations in the excretion of tyrosine metabolites."

Medical College of Virginia
Richmond, Va.

288. JAMES G. W., III, and ABBOTT L. D., JR. *Studies on nitrogen and phosphorus metabolism with relation to the hematologic response in pernicious anemia patients after crystalline vitamin B_{12}*, Am. J Med. 11 521, Oct. 1951 (In Soc. Proc.)

"In view of the response in hemoglobin synthesis caused by crystalline vitamin B_{12} in patients with pernicious anemia it was considered desirable to correlate hematologic observations with nitrogen and phosphorus balance studies before and during clinical remission in an attempt to elucidate further the metabolic effects of this substance. Four previously untreated patients with pernicious anemia were studied for several weeks on a constant diet. Positive nitrogen balance as great as 6 gm. per day was produced by vitamin B_{12} in each case. When negative nitrogen balance existed during the control period, it promptly became positive. Individual patients made from 18 to 37 gm. of hemoglobin per day for an average period of eleven days. Nitrogen required for hemoglobin formation alone equalled or exceeded the total nitrogen retained during the corresponding period.

"The following sequence of biochemical changes was observed First, a prompt and striking decrease in urinary phosphorus which preceded any change in reticulocyte count second, increased excretion of uric acid during the upswing of reticulocytes and third, increased excretion of phosphorus during the period of greatest reticulocytosis. These clinical observations suggest that among the first metabolic effects of vitamin B_{12} is a profound influence on nucleoprotein synthesis."

Richmond, Va.

289 JAMES, G W., and ABBOTT L. D., JR.: *Metabolic studies in pernicious anemia. I Nitrogen and phosphorus metabolism during vitamin B_{12}-induced remission*, Metabolism 1 259-270, May 1952.

Authors summary of their observations on 4 patients

"1. Positive nitrogen balance was found to exist, as much as 6.0 gm. per day during remission induced by vitamin B_{12} in pernicious anemia. Nitrogen accumulated as blood proteins exceeded the dietary intake of nitrogen, indicating conversion of tissue nitrogen to blood proteins.

"2. Urinary phosphorus followed a pattern of rapid diminution after therapy then an increased excretion during reticulocytosis, and a gradual return to a normal excretion. This was in part responsible for a positive phosphorus balance immediately after therapy a negative balance during reticulocytosis, and then a positive phosphorus balance.

"3. The decrease in the urine phosphorus excretion is one of the earliest biochemical alterations we have observed in therapy induced remission and is probably related to a fundamental effect of vitamin B_{12} on nucleoprotein metabolism occurring in the change from a megaloblastic to an erythronormoblastic marrow

"4. Uric acid excretion increased with reticulocytosis and is considered a reflection of accelerated nucleoprotein metabolism."

Medical College of Virginia
Richmond, Va.

FECAL CONTENT OF VITAMIN B_{12}

290. CALLENDER, S. T E., MALLETT B. J., SPRAY, G. H., and SHAW G E. *Anti-anaemia activity of faecal extract from pernicious anaemia patient preliminary communication*, Lancet 2 57 July 9 1949

A fecal extract was obtained from a patient with pernicious anemia, which on assay with Lactobacillus lactis Dorner had activity equivalent to 1 mcg. of vitamin B_{12} per cc. It produced an optimal reticulocyte response and a rise in hemoglobin in a patient with untreated pernicious anemia when it was administered intramuscularly in a daily dosage of 5 cc. for five days. Also, the marrow of the patient, which previously had been megaloblastic, became definitely normoblastic, and subjective symptoms of subacute combined degeneration of the central nervous system improved. A chromatogram of the extract closely resembled chromatograms of purified parenteral liver extracts, which suggests that the anti-pernicious anemia activity of the fecal extract was due to vitamin B_{12}.

291 GIRDWOOD R. H.: *The intestinal content in pernicious anemia of factors for the growth of Streptococcus faecalis and Lactobacillus leichmannii*, Blood 5: 1009-1016, Nov 1950.

During a study of 3 cases of untreated pernicious anemia and 2 control patients, it was found that the gastric juice and small intestinal secretions obtained during a period of fasting contained only very small amounts of folic acid or the growth factors for Lactobacillus leichmannii, which include vitamin B_{12}. No evidence was found of a consistent increase or decrease of these factors along the intestine.

Both folic acid and vitamin B_{12} appear to be synthesized in relatively large amounts in the large intestine. The daily output in the stools may approximate 5 mcg. of vitamin B_{12} and 0.5 mg of folic acid in patients with pernicious anemia as well as in control patients.

The author concludes from his results that folic acid and vitamin B_{12} are produced by bacteria in the large intestine of man, apparently at least as well in pernicious anemia patients as in normal subjects. It is thought unlikely that relapses and spontaneous remissions in pernicious anemia can be associated with changes in the intestinal flora of the small intestine, leading to the destruction or formation of significant amounts of folic acid or vitamin B_{12} at the levels of maximum absorption. However the author states that the data presented do not entirely rule out such a mechanism.

In an addendum is reported the rectal administration to a pernicious anemia patient of 100 cc. of normal gastric juice together with 25 mcg. of vitamin B_{12} daily for four days. No beneficial effect was observed. The administration of 100 cc. of normal gastric juice by mouth together with 25 mcg. of vitamin B_{12} daily for four days produced a reticulocytosis of 8.4 per cent and a rise of red cells from 2,640,000 to 3,170,000 per cu. mm. over a period of 20 days.

Thomas Henry Simpson Memorial Institute for Medical Research
Ann Arbor Mich.

292. CALLENDER S T E., and SPRAY G H: *Preparation of haemopoietically active extracts from faeces*, Lancet 1 1391 1392, June 30 1951

In experimental studies extracts were prepared from the feces of a normal person and from 2 patients with untreated pernicious anemia. This material produced improvement when given by intramuscular injection to patients with addisonian anemia in relapse The authors state that the active substance in the extracts appears to be B_{12}, and seems to be similar in amount in a normal person and in test patients with untreated pernicious anemia.

Radcliffe Infirmary
Oxford, England

CLINICAL TEST FOR TOXIC REACTION

293. BEDFORD P D *Side-effects of a preparation of vitamin B_{12}*, Brit. M. J 1 690-691 March 29 1952.

In an effort to determine whether vitamin B_{12} itself or a contaminant was responsible for side effects noted, three vitamin B_{12} preparations were tested on 100 people. None reacted adversely to a solution of vitamin B_{12} derived from liver and containing 10 mcg of the vitamin per cc.

Fourteen people were skin-test positive to a proprietary preparation containing B_{12} 20 mcg./cc. and derived by customary extraction procedures from Streptomyces fermentation liquors. Six suffered side effects when this solution was given intramuscularly

Two people were skin-test positive to another similar proprietary preparation except that it was "purified by crystallization and therefore likely to contain a smaller proportion of any impurity" None were sensitive to its intramuscular use. Impurities carried over from the mold fermentation liquor were regarded as responsible for the sensitivity phenomena. Both cutaneous reactions and side effects were found to be more than twice as common in people who had previously been treated with antibiotics. The author advanced the possibility of inducing idiosyncrasy to antibiotics as a reason against the use of impure preparations of vitamin B_{12} derived from mold cultures.

Cowley Road Hospital
Oxford, England

ANIMAL STUDIES

TOXICITY STUDIES

294. WINTER, C. A., and MUSHETT C. W *Absence of toxic effects from single injections of crystalline vitamin B_{12}*, J Am. Pharm. A. (Sc. Ed.) 39 360-361, June 1950.

The authors administered crystalline vitamin B_{12} to mice in doses of 100, 200, 400 and 1600 mg. per kg. intraperitoneally and in doses of 800 and 1600 mg. per Kg. intravenously In addition, the vitamin was injected intraperitoneally in rats and guinea pigs in doses up to 100 mg. per kg. In no instances were there any deaths or toxic manifestations. Some of the mice were sacrificed 48 hours or one week after the injection was given, and no gross pathologic changes were observed at autopsy These results differ from those of Traina (Arch. Path. 49 278, 1950 [Abstr 295]) who described toxic reactions and deaths in mice treated with a concentrate containing vitamin B_{12}: intraperitoneal or subcutaneous injections of 3 mg. per Kg. of B_{12} in this form produced death in all of the treated animals. Since the largest doses of the crystalline vitamin used by the present authors was more than 500 times as great as those of the B_{12} concentrate used by Traina, it appears that the toxic manifestations observed by him were probably due either to impurities in the preparation, or to contamination of the sample. On the basis of the present results crystalline vitamin B_{12} is essentially a nontoxic substance.

Merck Institute for Therapeutic Research
Rahway N J

295. TRAINA, V *Toxicity studies on vitamin B_{12} in albino mice* Arch. Path. 49 278-279 March 1950.

It was found that in the albino mouse an intraperitoneal dose of about 0.75 mg./Kg. of the vitamin B_{12} preparation used is not toxic an intraperitoneal dose of 1.5 mg./Kg. is decidedly toxic and a dose of 3 mg./Kg., given either intraperitoneally or subcutaneously is lethal for 100 per cent of the animals treated. Congestion and edema of the lungs and slight congestion of the visceral organs were the only pathologic changes observed in the animals that died.

In a separate study it was found that guinea pigs showed no sensitivity to vitamin B_{12}. The author cites unpublished data of his own which show that vitamin B_{12} possesses antianaphylactic and antihistaminic activity

Fairview Park Hospital
Cleveland, Ohio

ANEMIAS; OTHER BLOOD STUDIES

Chicks

296. WAGLEY P F., and MORGAN, H. R. *Observations on the effects of folic acid antagonists, folic acid, liver extract and vitamin B_{12} on embryonated eggs a preliminary report*, Bull. Johns Hopkins Hosp. 83: 275-278, Sept. 1948.

Chick embryos were inoculated via the yolk sac with three folic acid antagonists, alone, or 24 hours after folic acid, liver extract, or vitamin B_{12} had been introduced into the sac. 4-Amino-pteroylglutamic acid in amounts of 0 005 mg. decreased the survival time of the embryos and produced cytologic changes in the yolk-sac blood islets (diminution in the size and number with karyolysis and karyorrhexis of many of the remaining nuclei) This effect was not altered by the injection of 0.005 mg. of vitamin B_{12} simultaneously with the inhibitor or by the injection of 3.75 U.S.P units of purified liver extract (with activity equivalent to that of 2.7 micrograms of vitamin B_{12}) However when 12.5 mg. of folic acid was injected 24 hours prior to the antagonist the blood islets

were not decreased in number although their cellularity was somewhat diminished. Pyknosis, karyolysis and karyorrhexis of the nuclei were not present. There was a suggestive increase in survival time.

The changes produced by methyl-4-amino-pteroyl glutamic acid, which were similar to those produced by 4-amino-pteroylglutamic acid but less marked, were altered by folic acid but not by liver extract.

N^{10}-methyl-pteroic acid caused no detectable changes in the yolk sac blood islets.

297 WAGLEY P F., and MORGAN, H. R. *Effects of folic acid antagonists inoculated in embryonated eggs*, Arch. Path. 46 441-450, Nov 1948.

Chick embryos were inoculated via the yolk sac with three folic acid antagonists, alone or 24 hours after folic acid, liver extract, or vitamin B_{12} had been introduced into the sac. 4-Aminopteroylglutamic acid caused pronounced changes in the hemopoietic tissue of the embryos decrease in the number size, and cellularity of the blood islets of the yolk sac and pyknosis and nuclear fragmentation of the cells that remained. Methyl-4-aminopteroyl glutamic acid in comparable doses had much less effect on the blood islets, and N^{10}-methylpteroic acid in relatively large doses had no appreciable effect. The effects of 4-aminopteroylglutamic acid were counteracted by folic acid, but unaltered by liver extract or by vitamin B_{12}. The survival time of embryos inoculated with 4-aminopteroylglutamic acid decreased with increasing dosage of the antagonist. The technics employed in this study are described the yolk sac method is a new way of evaluating the effects of folic acid inhibitors.

Boston City Hospital, and
Harvard Medical School
Boston, Mass.

298. NICHOL, C. A., HARPER, A. E., and ELVEHJEM, C. A. *Effect of folic acid, liver extract, and vitamin B_{12} on hemoglobin regeneration in chicks* Proc. Soc. Exper Biol. & Med. 71: 34-37 May 1949 Federation Proc: 8 233, March 1949

Severe anemia was induced in chicks by the intramuscular injection of phenylhydrazine hydrochloride following a depletion period on a folic acid-deficient diet. The effect of folic acid, liver extract, and vitamin B_{12} on the rate of hemoglobin regeneration in these anemic chicks was studied.

Liver extract alone did not influence the rate of hemoglobin formation. The combination of liver extract and folic acid caused a more rapid regeneration of hemoglobin than could be obtained with folic acid alone. Vitamin B_{12} completely replaced liver extract in stimulating the formation of hemoglobin in the presence of folic acid.

Thus it appears that the component of liver extract causing the stimulation of hemoglobin regeneration in the presence of folic acid is vitamin B_{12}. The metabolic interrelationship between folic acid and vitamin B_{12} is not known. It may be that the vitamin makes more folic acid available to the animal. However it may also act directly in the normal mechanism of hemoglobin formation, as is implied by its activity in patients with pernicious anemia. The results of the present study indicate that vitamin B_{12} does not have optimum activity on hemoglobin regeneration in chicks unless folic acid is present in adequate amounts.

299 DE GUIA, E. F., and WOLFRED M. M.: *Comparative hemopoietic and growth factor responses to vitamin B_{12}, folic acid and iron*, J Am. Pharm. A. 60 299-301 June 1951.

The authors report further investigations on the response of the hemopoietic and growth factors in anemic chicks to vitamin B_{12}, folic acid, and iron. The basal diet included methionine, 0.3 Gm. thiamine, 0.4 mg.; riboflavin, 0.8 mg. nicotinic acid, 5.0 mg.; pyridoxine, 0.6 mg.; calcium pantothenate, 2.0 mg. biotin, 0.02 mg. choline chloride, 200 0 mg ; inositol, 100.0 mg.; a-tocopherol, 0.50 mg. vitamin K_1 2.50 mg. Chicks became anemic (red blood cells 2 million per cu. mm. and hemoglobin 5 Gm. or less per 100 cc.) in 24 to 30 days. The mortality rate before anemia was reached was 26.6 per cent. Anemic chicks showed body tremors, extended wings and poor balance, signs of paralysis, and poor growth and feathering.

Vitamin B_{12} supplements did not cause significant improvement in red blood cell and hemoglobin levels. Parenteral administration of B_{12} in doses of 6 mcg. produced greater growth, while chicks receiving 3 mcg. per 100 Gm. of feed died within ten days after treatment was begun. No improvement in feathering was noted.

All the chicks given daily supplements of 6 mg. of folic acid parenterally showed rises in red cell counts and hemoglobin levels, but normalcy was not reached. These chicks showed a greater weight increase than those which received B_{12}, and normal feathering returned.

Chicks receiving 6 mcg. B_{12} plus 6 mg. of folic acid showed the greatest increase in weight. Blood counts and hemoglobin levels returned to normal after nine parenteral injections or after 12 days of oral administration. Normal feathering began to appear within two days.

Chicks treated with 3 mg. ferric ammonium citrate plus 3 mcg. of B_{12} given parenterally showed slightly greatly weight increases than those treated with B_{12} alone. However a hemopoietic response comparable to that produced by 6 mg. of folic acid plus 6 mg. of ferric ammonium citrate was produced by 6 mcg of B_{12} plus 6 mg. ferric ammonium citrate. No improvement in feathering was noted.

Chicks treated with folic acid and iron parenterally or orally showed greater weight gains than all others and higher red cell and hemoglobin levels than those receiving folic acid alone, but did not achieve normalcy Normal feathering developed within four days.

Thus, of all supplements tested, only the combination of folic acid and B_{12} cured the anemia. There is evidence that B_{12} may function somewhat as a catalyst by facilitating the uptake or release of folic acid by body tissue.

University of Southern California
Los Angeles, Calif.

300. STERN J R., HSU J M., and McGINNIS, G: *Vitamin B , and hemoglobin regeneration in the chick*, J Biol. Chem. 194 191 196, Jan. 1952.

The effect of vitamin B_{12} deficiency upon hemoglobin formation in the chick was studied. Four groups of from 12 to 30 chicks each, hatched from hens receiving a B_{12}-deficient ration were fed a corn-soybean oil meal basal fortified with B-vitamins, vitamins A and D and terramycin (6.8 mg./lb.) Two groups received 30 mcg. of crystalline B_{12} per kg. of diet. Two mg. of phenylhydrazine hydrochloride per 100 Gm. of body weight was injected subcutaneously on alternate days into one of the B_{12}-supplemented lots and one on the basal alone. The phenylhydrazine administration was begun when the chicks were 13 days old in a second trial the drug was given at 6 days of age.

Unlike the rat, mouse, pig fox, and mink, the chick was able to maintain normal hematocrit and hemoglobin levels in an uncomplicated vitamin B_{12} deficiency although the growth rate was severely retarded When the vitamin B_{12} deficiency was aggravated by an anemia resulting from injections of phenylhydrazine hydrochloride, vitamin B_{12} was shown to have a stimulatory action on hemoglobin and hematocrit. In the presence of phenylhydrazine, chicks fed the B_{12} had significantly higher hemoglobin, hematocrit, and erythrocyte concentrations than chicks deficient in B_{12}. A single injection of phenylhydrazine into 3 week old chicks caused anemia, and a seven day period elapsed before the hemoglobin level of deficient chicks reached a normal level, whereas only five days were required for hemoglobin of vitamin B_{12}-supplemented chicks to return to normal.

Terramycin, which was fed to all groups and which stimulated growth of chicks on a complete diet, did not replace B_{12} as a growth stimulant.

Washington State College
Pullman, Wash.

Mice

301. VIJAYARAGHAVAN P K., and DUNN M. S. *Effect of crystalline vitamin B_{12} on experimental anemia in mice* Proc. Soc. Exper Biol. & Med. 75: 754-756, Dec. 1950.

Crystalline vitamin B_{12} injected intraperitoneally into mice with anemia experimentally induced by phenylhydrazine increased the number of red blood cells per unit volume, but not the hemoglobin concentration. Prior to and during the experiment the mice were kept on a chemically defined diet probably containing a relatively small amount of vitamin B_{12} but adequate amounts of other hematopoietic substances. The increase in red blood cells during the first four days of severe anemia was found to be directly proportional to the amount of vitamin B_{12} administered. These results suggest the possible development of an assay procedure for the determination of vitamin B_{12}.

University of California
Los Angeles Calif

302. VIJAYARAGHAVAN P K., and DUNN., M. S.: *Effect of vitamins B_{12}, B_{12a} and B_{12b} on red blood cell counts in experimental anemia in mice* Arch. Biochem. 31: 248-250, April 1951.

The potencies of vitamins B_{12}, B_{12a}, and B_{12b} in raising the red blood cell counts in anemic mice were tested. Anemia was produced by a single intraperitoneal injection of phenylhydrazine hydrochloride, 6 to 8 mg. per 100 Gm. of body weight. This dosage had been found to reduce the red blood cell count to 50 per cent of its normal value. Following injection of 2.00 to 2.16 mcg. daily of several manufacturers vitamin B_{12} preparations for two or four days after the peak of anemia had been reached, the red blood cell count in those animals that received B_{12} was more than twice that of the control mice. Increases averaged 0.89 millions per cu. mm. after two days of treatment, and 0.30 millions per cu. mm. after four days. Differences among the various B_{12} preparations were not significant. The authors assume, therefore, that these B_{12} preparations are similar if not identical, in composition and structure of the chemical groups responsible for the effects noted.

University of California
Los Angeles, Calif.
Nutrition Research Laboratories
Coonoor India

303 VIJAYARAGHAVAN P., and DUNN M.: *Effect of thymidine, citrovorum factor folic acid and degradation products of vitamin B_{12} on red blood counts in experimental anemia in male mice* Arch. Biochem. & Biophysics 36 299-303, 1952 (abstr Blood 7 856-857 Aug. 1952)

"The hematopoietic effect of various agents was tested on male mice following the peak of anemia induced by intraperitoneal injection of phenylhydrazine hydrochloride. The red blood cell counts determined under these conditions were increased comparably by injections of the citrovorum factor at high levels (200 mg.) and vitamin B_{12} at low levels (2 mg.) The compounds thymidine, alpha ribazole, 5,6-dimethylbenzimidazole, 1-D-ribityl-5,6-dimethylbenzimidazole, and 1,2-diamino-4,5-dimethylbenzene exhibited little or no antianemic activity while the synthetic analog (2,5-dimethylbenzimidazole) of these degradation products of vitamin B_{12} inhibited red blood cell formation to a level below that obtained on a folic acid-free diet."

University of California
Los Angeles, Calif.

Rats

304 CAMERON D G., CALLENDER, S. T E., WATSON G M., and WITTS, L. J *Experimental macrocytic anaemia in the rat treated with purified liver extract, pteroylglutamic acid and vitamin B_{12}*. Nature 164 188 July 30, 1949 (Letter to Editor)

Pteroylglutamic acid prolonged the survival time of rats in which experimental macrocytic anemia had been produced by formation of a cul-de-sac in the small intestine, whereas purified liver extract and vitamin B_{12} did not. Hematologic remissions occurred with all three substances, but more frequently with pteroylglutamic acid. One control animal had a spontaneous remission.

Radcliffe Infirmary
Oxford England

305. KODICEK, E., and CARPENTER, K. J: *Experimental anemias in the rat. II The effect of various sulfonamides in producing a pteroylglutamic acid deficiency and the pteroylglutamic acid activity of test substances*, Blood 5 540-552, June 1950.

Authors summary "Different sulfonamides were tested to ascertain their effect in producing the character symptoms of acute PGA deficiency in rats fed on synthetic diets. Sulfasuxidine (1 per cent) and the less soluble phthalylsulfathiazole (1 per cent) were equally effective. Sulfathiazole in 1 per cent concentration produced a hemolytic anemia not reversible by PGA or whole liver powder In a 0.5 per cent concentration it was also effective, but in view of its toxicity the less soluble sulfonamides were to be preferred. A mixture of 0.5 per cent sulfathiazole and 0.5 per cent sulfadiazine was extremely toxic and produced a hemolytic anemia. Sulfaguanidine was toxic at 1 per cent concentration.

"When intermittent small doses of PGA were given to PGA-deficient rats to prolong their life from 45 up to 155 days, 1 per cent sulfasuxidine or phthalylsulfathiazole, or 0.5 per cent sulfathiazole were equally efficient in producing regularly a macrocytic normochromic anemia.

"The response of PGA-deficient rats to single doses of PGA has been studied and an assay procedure has been suggested which uses the weight increase and duration of cure as the measure of the response. The W.B.C. and reticulocyte response can also be used as a qualitative indication of PGA activity

"Of the substances tested by this procedure, vitamin B_{12}, purified pernicious anemia preparations, ascorbic acid and xanthopterin showed no PGA activity A commercial yeast preparation and Teropterin were found to possess biologic activity comparable with that found by other workers in assays on chicks."

University of Cambridge, and Medical Research Council Cambridge, England

306. BORSON H. J., SINGMAN D., LEPKOVSKY S., DIMICK, M. K., GASC, V., and PERRY R.: *Hematologic changes and death in vitamin B_{12}-deficient rats* Am. J Physiol. 162 714-720, Sept. 1950.

A high mortality was observed within three weeks after weaning in rats weaned on vitamin B_{12}-deficient diets. Death was associated with a leukopenia and granulocytopenia which were not per se the causes of death. It is suggested that these were manifestations of a profound biochemical or anatomic lesion which was the actual cause of death. Vitamin B_{12} deficiency possibly is associated with disturbed protein metabolism.

Some of the animals were given crystalline vitamin B_{12} or vitamin B_{12} concentrate in doses of 1 mcg. subcutaneously twice a week. In all except the moribund animals this treatment resulted in prompt improvement—recovery from leukopenia, granulocytopenia, anemia, and rapid and sustained weight gain. No difference was observed in the effects of the two vitamin B_{12} preparations.

A few animals were treated with the sodium salt of folic acid (0.5 mg. subcutaneously twice a week) Results were favorable in some of these, but were not nearly so consistent or spectacular as results obtained with vitamin B_{12}.

Some of the animals recovered spontaneously This was thought to be due, perhaps, to adaptive mechanisms, to changes in vitamin B_{12} requirements, or to the availability of vitamin B_{12} from intestinal synthesis.

University of California Medical School Berkeley Calif
University of California San Francisco, Calif

307 JÜRGENS, R., and STUDER, A.: *Experimental leukopenia in rats and their therapeutic response to vitamins metals and other substances*, Acta haematologica 5 47-64, 1951 (abstr Blood 7 382, March 1952)

"Succinylsulfathiazol was fed to 1,000 rats in order to produce a striking granulocytopenia. This was used as a test for the examination of the leukopoietic effect of various substances. Especially effective was folic acid, even in experiments with additional damage of the bone marrow by nitrogen mustard. Vitamin A and C and iron had a similar leukopoietic effect. No response was seen using the vitamins B_2, B_6, B_{12}, E, the nucleic acid derivatives hypoxanthindesoxyriboside, guanidindesoxyriboside thymin, thymidine, uracil, furthermore [sic] choline, testosteronpropionate, methionine and thyrosine."

Hoffmann-La Roche Co. Basle, Switzerland

308. WATSON G M., and WITTS, L. J *Intestinal macrocytic anaemia*, Brit. M. J 1 13-17 Jan. 5, 1952.

Megaloblastic anemia was induced in rats by forming a blind loop in the middle third of the intestines. The anemia was associated with hemolysis and sometimes with steatorrhea. Response to folic acid or aureomycin was good, but to vitamin B_{12}, poor or absent. With reference to the mechanism of the experiment the authors state: "From the available evidence the most likely cause of the anaemia is an alteration of the intestinal flora analogous to that which occurs in cases of gastro-colic fistula in man, and it is possible that the intestinal bacteria are in some way concerned with the absorption or utilization of haemopoietic substances."

Radcliffe Infirmary Oxford, England

309 CRAFTS, R. C. *The effects of cobalt, liver extract, and vitamin B_{12} on the anemia induced by hypophysectomy in adult female rats*, Blood 7 863-873, Sept. 1952.

Author s summary: "Hypophysectomized adult female rats of the Wistar strain were treated with daily subcutaneous injections of 0.1 0.3 and 0.5 mg. of cobalt nitrate for 50 days. Most striking results were obtained with the largest dose. The erythrocyte count in the hypophysectomized rats was elevated from a preoperative level of 7.9 million cells per cu. mm. to 11.6 million cells; the hematocrit, from 45.8 per cent to 60.8 per cent; and the hemoglobin, from 16.0 Gm. per 100 cc. to 21.0 Gm. Normal animals respond in a similar manner Cobalt nitrate, therefore, not only prevented anemia but actually induced a marked elevation in the number of erythrocytes and in hemoglobin. Vitamin B_{12} [1 or 2 mcg. daily subcutaneously] and liver extract were ineffective."

University of Cincinnati College of Medicine Cincinnati, Ohio

Swine

310. HEINLE, R. W., WELCH, A. D., and PRITCHARD J A.: *Essentiality of both the antipernicious anemia factor of liver and pteroylglutamic acid for hematopoiesis in swine,* J Lab. & Clin. Med. 33 1647 Dec. 1948 (In Soc. Proc.); Proc. Central Soc. Clin. Res. 21 94 1948.

Cleveland, Ohio

311 CARTWRIGHT G E., and WINTROBE, M. M. *Experimental production of a nutritional macrocytic anemia in swine* Federation Proc. 8 351-352, March 1949

A nutritional macrocytic anemia was produced in 32 swine fed a diet deficient in pteroylglutamic acid. This was not prevented by the presence of "extrinsic factor" in the diet. Purified liver extracts as well as vitamin B_{12} possessed some hemopoietic activity under the conditions employed, but less than pteroylglutamic acid compounds.

312. CARTWRIGHT G E., TATTING B., ASHENBRUCKER, H., and WINTROBE, M. M.: *Experimental production of a nutritional macrocytic anemia in swine* Blood 4 301-323, April 1949

A macrocytic anemia, similar morphologically to pernicious anemia and the related macrocytic anemias in man, was induced in swine made deficient in pteroyl glutamic acid by a purified diet containing casein (10 or 26%) and supplemented with B vitamins, sulfasuxidine. and a crude methyl folic acid antagonist. Administration of pteroylglutamic acid compounds caused a rapid return of both blood and bone marrow to normal. Purified liver extracts and crystalline vitamin B_{12} showed some hemopoetic activity in several animals.

313. PALMER, J G., CARTWRIGHT G. E., and WINTROBE, M. M. *Experimental production of a nutritional macrocytic anemia in swine,* Am. J Med. 8 541-542, April 1950 (In Soc. Proc.)

A folic acid deficiency characterized by macrocytic anemia was produced in swine. This deficiency was corrected by the administration of pteroylglutamic acid and some of its derivatives, but was only partially corrected by purified liver extract, vitamin B_{12}, ascorbic acid, and other substances. The administration of thymine and vitamin B_{12} together did not substitute for pteroylglutamic acid.

Experiments with 2,6-diaminopurine showed that it produced a metabolic deficiency differing from that produced by the "crude" methyl folic acid antagonist used in the first experiments.

Attempts were made to produce a deficiency of vitamin B_{12} in swine by raising young pigs on soybean protein rather than on a casein diet. Pteroylglutamic acid, sulfasuxidine and, in certain instances, desiccated thyroid were included in the diet. Following the administration of crystalline vitamin B_{12} to such animals a growth response was observed. The deficiency syndrome, however was not accompanied by significant anemia or striking alterations in the bone marrow

University of Utah College of Medicine
Salt Lake City Utah

314 CARTWRIGHT G E., PALMER, J G., TATTI
B., ASHENBRUCKER, H., and WINTROBE, M
Experimental production of nutritional macro
anemia in swine. III Further studies on pte
glutamic acid deficiency J Lab. & Clin. Med.
675-693, Nov 1950

In their summary the authors point out that experimental anemia in swine which is described has more similarities to refractory megaloblastic ar achrestic anemia in man and to many instances of tro macrocytic anemia, megaloblastic anemia of infancy pernicious anemia of pregnancy than to addisonian nicious anemia."

University of Utah College of Medicine
Salt Lake City Utah

315 CARTWRIGHT G. E., TATTING B., ROBINS
J., FELLOWS, N M., GUNN F D., and V
TROBE, M. M. *Hematologic manifestation*
vitamin B_{12} deficiency in swine, Blood 6 867
Oct. 1951

Of 39 pigs not receiving B_{12}, 13 failed to dev anemia, 16 developed a mild anemia and 10 devel a moderately severe anemia. The anemia when presen normocytic and in 24 pigs was accompanied by a erately severe neutropenia. There was increase in no blasts in sternal marrow Hematologic alterations neither consistently nor completely corrected by th ministration of vitamin B_{12} in spite of the fact that nite and marked reticulocyte increases followed.

These results are in contrast to those obtaine pigs with an experimentally produced deficiency of Such animals develop macrocytic anemia, leukopenia a macronormoblastic type of bone marrow

Megaloblastic anemia may not have developed i B_{12}-deficient pigs because (1) the deficiency was sufficiently severe to result in such a change, or because PGA prevents the development of megalobl anemia even in the absence of vitamin B_{12}.

316. CARTWRIGHT G E., and WINTROBE, M.
Experimental production of nutritional macro
anemia in swine, Am. J Physiol. 167 773-774
1951 (In Soc. Proc.)

"A severe macrocytic anemia, leukopenia and thrombocytopenia have been produced in 56 swine deficient in pteroylglutamic acid. The bone marrow such animals were hyperplastic and there was an inc in large immature nucleated red cells (macrono blasts) of a type which resembled but which were identical with the megaloblasts of pernicious anemia. hematologic manifestations of this deficiency appe in the presence of extrinsic factor in the diet and not prevented or alleviated by the administration of fied liver extract or crystalline vitamin B_{12}. In an to produce a deficiency of vitamin B_{12} a total of 70 were fed a purified diet containing soybean alpha pr in place of casein. Growth of the animals on the diets without added vitamin B_{12} was retarded as comp with the growth of animals on the same diet with this vitamin. Anemia was not a prominent of the deficiency syndrome and when present was

mild in degree and normocytic in type. Abnormalities in the nucleated red cells in the sternal marrow were not present. A combined deficiency of both pteroylglutamic acid and vitamin B_{12} has been produced in 21 pigs. These animals developed a severe macrocytic anemia, leukopenia and slight thrombocytopenia. Pernicious anemia-like megaloblasts were observed in small numbers in the bone marrow The majority of the nucleated red cells were macronormoblasts. The anemia responded partially to crystalline vitamin B_{12} and completely to pteroylglutamic acid. The role of pteroylglutamic acid and vitamin B_{12} in normal erythropoiesis is discussed."

University of Utah College of Medicine Salt Lake City Utah

317 CARTWRIGHT G E., TATTING B., KURTH, D., and WINTROBE, M. M.: *Experimental production of nutritional macrocytic anemia in swine V Hematologic manifestations of a combined deficiency of vitamin B_{12} and pteroylglutamic acid*, Blood 7 992-1004, Oct. 1952.

Authors' summary "A total of 20 swine were fed a diet adequate in all known respects except that soybean protein was substituted for casein, succinylsulfathiazole and a folic acid antagonist were added, and vitamin B_{12} and pteroylglutamic acid were withheld from the vitamin supplement.

"The animals developed macrocytic anemia, leukopenia and neutropenia, accompanied by erythroid hyperplasia of the bone marrow The erythroblasts consisted mainly of immature macronormoblasts but a few atypical megaloblasts were also observed.

"The anemia responded rapidly and completely to the administration of both vitamin B_{12} and pteroylglutamic acid. The administration of pteroylglutamic acid alone resulted in an immediate return of the blood and bone marrow to within normal limits but after several months there was a partial hematologic relapse in spite of continued therapy with this vitamin. The administration of vitamin B_{12} alone resulted in only partial remission of the anemia and the bone marrow remained macronormoblastic although the megaloblasts tended to disappear

"Growth of the animals was stimulated by the administration of either vitamin but the administration of both vitamins simultaneously resulted in the greatest rate of growth.

"No manifestations of neurologic disturbances or of increased pigment excretion were observed in the deficient swine."

University of Utah College of Medicine Salt Lake City Utah

Dogs

318. RUEGAMER, W R., BRICKSON, W L., TORBET N J., and ELVEHJEM, C. A. *Response of dogs to liver extracts containing the pernicious anemia factor* J Nutrition 36 425-435, Oct. 1948.

Liver extracts rich in pernicious anemia factor were effective in restoring the blood picture and general health of young dogs fed a niacin-deficient diet, containing 1% sulfasuxidine. Combination of liver extracts with folic acid produced complete recovery

Monkeys

319 MAY C. D., SUNDBERG R. D., SCHAAR, F., LOWE, C. U., and SALMON R. J *Experimental nutritional megaloblastic anemia relation of ascorbic acid and pteroylglutamic acid I Nutritional data and manifestations of animals* A.M.A. Am. J Dis. Child. 82 282-309 Sept. 1951

Authors' summary "Megaloblastic anemia has been produced regularly in monkeys by feeding milk diets deficient in ascorbic acid. Monkeys fed the same milk diets supplemented with adequate ascorbic acid remain in good health for prolonged periods and maintain normal blood and bone marrow This experimental megaloblastosis is virtually indistinguishable from that seen in megaloblastic anemia in humans, particularly the form encountered most frequently in infancy This experimental megaloblastosis can be eliminated or prevented by pteroylglutamic acid or folinic acid without the aid of ascorbic acid. It can also be cured by ascorbic acid alone. Vitamin B_{12} will neither cure nor prevent it. This experimental megaloblastosis is due to a disturbance in the metabolism of pteroylglutamic acid caused by a deficiency of ascorbic acid. Application of this knowledge to elucidation of the pathogenesis of megaloblastic anemia in infancy is mentioned."

University of Minnesota Medical School Minneapolis, Minn.

320. SMITH, S. C., and ELVEHJEM, C. A. *An interrelation of pteroylglutamic acid, vitamin B_{12} and the monkey anti-anemia factor* J Nutrition 45 47-59 Sept. 1951.

Authors' summary "A deficiency of PGA was created in young rhesus monkeys by means of a purified diet. Signs characteristic of a lack of the monkey anti-anemia factor accompanied those of the primary deficiency Remission of the monkey anti-anemia factor deficiency syndrome was obtained by the oral administration of PGA at a level 10 times the minimum daily requirement.

"Remission for a limited period was also obtained with a 60% methanol extract of liver supplying only 4 µg. each of PGA and vitamin B_{12} per day Eventually PGA had to be supplied to maintain normal growth rate and blood picture, but it was required in much smaller amounts than when the methanol extract of liver which is a good source of the monkey anti-anemia factor was not employed.

"Vitamin B_{12} produced responses only in the less-deficient monkeys and probably acted in conjunction with vitamin C to stimulate the synthesis of PGA.

"A relationship exists among PGA, vitamin C, vitamin B_{12} and the monkey anti-anemia factor although the latter two substances are not ordinarily required in the diet of the normal monkey

"At high levels PGA appears to be capable of satisfying any requirement that may exist in a PGA-deficient monkey for vitamin B_{12} or the factor or factors present in methanol extract of liver (the monkey anti-anemia factor)"

University of Wisconsin Madison, Wis.

321. POPPEN K. J., GREENBERG L. D., and RINEHART J. F. *The blood picture of pyridoxine deficiency in the monkey.* Blood 7: 436-444, April 1952.

Authors summary and conclusions: "Pyridoxine deficiency in the monkey results in a severe anemia characterized by hypochromia, increase in mean diameter decrease in mean cell thickness, dehydration, the appearance of 'target' cells and increased resistance to hemolysis.

"The administration of pyridoxine results in restoration of hematologic values to normal, correction of dehydration and disappearance of 'target' cells.

"Further evidence is offered relating 'target' cells to dehydration and, in addition, possibly to hepatic damage.

"A delayed macrocytic anemia which was not corrected by low doses of vitamin B_{12}, folic acid or iron is described in control animals. This anemia occurred chronologically one to two years later than the induced anemia of pyridoxine deficiency and is not necessarily related. The cause of this anemia is unknown.

"Normal hematologic data on twenty-one M. rhesus monkeys is presented."

University of California School of Medicine
San Francisco, Calif.

322. MAY C. D., HAMILTON, A., and STEWART, C. T.: *Experimental megaloblastic anemia and scurvy in the monkey. IV. Vitamin B_{12} and folic acid compounds in the diet, liver, urine and feces and effects of therapy.* Blood 7: 978-991, Oct. 1952.

Authors conclusions: "The concentration of vitamin B_{12} and folic acid compounds in the diet, liver, urine and feces of control and scorbutic monkeys was determined and compared with the type of hematopoiesis in the bone marrow.

"Monkeys fed milk diets devoid of ascorbic acid regularly developed a marked deficiency of folic acid compounds and megaloblastic anemia as a complication of scurvy.

"Megaloblastosis in the marrow did not develop in the scorbutic monkey unless the level of free folinic acid in the liver was low.

"Low levels of free folinic acid in the liver were found only with low levels of total folic acid, but the level of free folinic acid was sometimes found to be normal although the level of total folic acid was low.

"The fecal content of vitamin B_{12} and folic acid compounds was the same in control and scorbutic monkeys.

"Urinary excretion of folic acid did not prove to be a practical index of tissue stores.

"The effects of various procedures and therapeutic substances on the bone marrow and the liver content of vitamin B_{12} and the folic acid compounds was studied.

"Treatment of the scorbutic megaloblastic monkey with either folic acid or L-ascorbic acid restored the marrow to normal and caused accumulation of folic acid and folinic acid in the liver.

"The effect of L-ascorbic acid was quite specific as D-ascorbic acid and D-glucoascorbic acid had no effect on the marrow or the liver concentration of folic acid compounds."

University of Minnesota College of Medical Sciences
Minneapolis, Minn.
State University of Iowa
Iowa City, Iowa

323. SUNDBERG R. D., SCHAAR, F., and MAY C. D. *Experimental nutritional megaloblastic anemia. II. Hematology.* Blood 7: 1143-1181, Dec. 1952.

Authors conclusions: "Megaloblastic anemia has been produced in monkeys by feeding milk diets, low in folic acid, and deficient in ascorbic acid.

"Vitamin B_{12} was not an effective therapeutic or prophylactic agent.

"Folic acid caused prompt reversion of the megaloblastic marrow to a normoblastic marrow. If ascorbic acid was also given, the monkey survived and the hemoglobin and erythrocyte levels returned to normal.

"Ascorbic acid with no source of folic acid other than the experimental diet caused reversion of the marrow from megaloblastic to normoblastic. The differential counts suggest that this process was slightly slower than the similar change which occurred with folic acid.

"Folinic acid proved an effective therapeutic agent in doses smaller than the doses of folic acid which proved effective in these experiments.

"Semi-starvation did not produce megaloblastic anemia.

"The transformation of the megaloblastic to the normoblastic marrow has been discussed.

"The type, sequence and rate of changes in the marrow pattern in response to various therapeutic agents has been illustrated, presented in graphic form and discussed."

University of Minnesota College of Medical Sciences
Minneapolis, Minn.

LEUKEMIAS

324. HEILMEYER, L. *"Perniciöse Anemie" als Initialphase einer akuten erythroleukämie (Pernicious anemia as the initial phase of acute erythroleukemia).* Schweiz. med. Wchnschr. 80: 1122-1124, Oct. 14, 1950.

Two patients with initial diagnoses of pernicious anemia are described. Liver extract therapy in one and Heparglandol (each ampul of which contains 15 mcg. of vitamin B_{12} and 10 mg. of folic acid) in the other were ineffective. Subsequent sternal puncture studies revealed that true myeloblastic leukemia had developed. The diagnostic difficulties in this type of patient are discussed. The author states that the initial megaloblastic anemia in these patients was not of the pernicious type, but

rather to be part of a malignant transformation of the blood picture, hence the lack of response to vitamin B_{12}.

University of Freiburg
Freiburg, Germany

325. BARNARD R. D., and FOX, H. L.: *Innocuity of protracted oral administration of special fermentation concentrates of B_{12} as demonstrated in a patient with acute leukemia*, Ohio State M. J 46 784-786, Aug. 1950.

The authors describe a patient with acute leukemia who was released from the hospital on Dec. 18, 1949 in anticipation of rapid deterioration and death. On the day of discharge, the following dietary supplement was instituted, to be taken four times a day: 20 Gm. of crude linseed oil emulsified in milk with Tween 80, and 10 Gm. of crude fermentation concentrates from an antibiotic-producing Streptomyces strain. The patient began to improve on this regimen and, as of April 1950, he apparently was normal both clinically and hematologically. During this period his daily dietary supplement contained 240 to 320 mcg. of vitamin B_{12} and 9 to 12 mg. of antibiotic (in terms of streptomycin activity). In April the linseed oil-Tween 80 emulsion was discontinued, and a concentrate from a non-antibiotic-producing Streptomyces strain was given in doses corresponding to 550 mcg. of vitamin B_{12} a day. Clinical, but not hematologic, deterioration followed. Thereafter the daily diet was supplemented by a concentrate supplying 80 mcg. of vitamin B_{12} and 3 mg. of antibiotic, and by 750 mg. of terramycin (from Streptomyces rimosus). As of July 1950, the patient was receiving 100 mg. of terramycin daily. He was now in the eighth month of remission.

Altogether the senior author has recorded 29 cases of acute leukemia in which dietary supplements from antibiotic-producing Streptomyces species have produced 18 remissions. The authors believe the remissions to be due to the coliform-suppressing antibiotic present in the fermentation concentrate. With regard to vitamin B_{12}, the remission in the case described was believed to be probably coincidental to its administration. The results, however, imply the complete innocuity of massive oral doses of vitamin B_{12}.

The authors consider that, in the acute leukoblastic process, the blast is a result rather than a cause of the disease. They do not use myelosuppressive therapy. They state that the tonic and supportive properties of factors associated with vitamin B_{12} may eventually play an important part in the management of malignant leukoblastoses.

Terrace Heights Hospital
Queens, N. Y.

326. BARNARD R. D.: *Streptomyces fermentation derivatives in acute leukemia*, Lancet 1 1157-1159 May 26, 1951.

The author reports his experiences in treating acute leukemia with a fermentation by-product of Streptomyces griseus, and describes 3 illustrative cases. The first patient, a 17 year old girl, was treated with penicillin for a "viral" pneumonia. When she did not respond, and the spleen became palpable, blood and marrow samples were taken which indicated acute myeloblastic leukemia. Streptomyces residue (with a vitamin B_{12} equivalence of 6 mcg. per gram, and 220 "units" of streptomycin, equivalence in terms of Bacterium coli suppression) was given, 40 Gm. divided into four daily doses. Parenteral penicillin was discontinued after 24 hours, with the patient much improved, with increased appetite and appearance of health. Temperature became normal, and the patient got out of bed within 72 hours after the treatment was begun. The patient refused the residue mixture about two weeks after treatment was begun, and a relapse occurred 48 hours after the treatment was stopped. Death, apparently due to a cerebral hemorrhage, occurred 19 days after therapy was discontinued.

The patient had been ingesting, in the residue mixture, about 0.3 mg. of B_{12} and 8 mg. of antibiotic daily. "Blast" forms had declined and platelets had increased markedly during residue therapy, though the erythrocyte level remained the same.

The second patient, a 12 year old boy, was also treated with penicillin for a "viral" pneumonia. When acute leukemia was diagnosed, 10 Gm. daily of Streptomyces residue containing 55 mcg. of B_{12} per Gm., but without the coliform suppressing factor, was given orally. The patient declined during the next 12 days, and 13 days after the residue treatment was begun, 1.5 Gm. daily of terramycin hydrochloride was substituted for the antibiotic-free residue. Two days later the temperature returned to normal, and six days afterwards the child went home in clinical remission. The child died later, possibly as the result of a transfusion.

It is possible that, since the child received over 0.5 mg. of vitamin B_{12} daily, the vitamin was stored, and its combined activity was responsible for the remission. The author feels, however, that the action of the antibiotic element against the increased number of proteolytic organisms in the intestinal tract was responsible for the remission.

The third patient, a 69 year old man, was hospitalized with acute prostatism, and later an acute lymphoblastic leukemia was diagnosed. Aureomycin administration, 1 Gm. daily, was begun on admission. Transurethral resection was performed the second week, and when 0.25 mg. of terramycin and 0.25 mg. of chloramphenicol daily were substituted for the aureomycin, bleeding stopped and fever abated. Stools became odorless, with a predominantly gram-positive intestinal flora. Eight months after the apparent remission, the patient was in the best of health. On the combined terramycin and chloramphenicol treatment, and later on 100 mg. of terramycin daily, the erythrocyte count climbed steadily toward normal. Numbers of lymphoblasts declined rapidly on the same treatment. In this case terramycin apparently eliminated an aureomycin-resistant strain of Bacterium aerogenes.

The three cases reported are illustrative of the 50 patients the author has treated. Convincing statistics on all the patients are lacking, since other factors, such as transfusions and anti folic acid treatment, confuse the picture. There were only six one-year survivals among 50 patients.

In an addendum, the author mentions that Kersey (*Proc. Soc. Am. Bact.*, March 20, 1951) reports survival

of intestinal lactobacillus with small doses of terramycin, but not with large doses.

Author's summary "Food supplements obtained by fermentation with Streptomyces griseus have been given by mouth to patients with acute leukaemia. In some cases the clinical condition has greatly improved, but the blood picture has not shown corresponding changes.

"Though the products used are rich in vitamin B_{12} their effect in leukaemia is more probably related to their content of antibiotic. Streptomyces-derived antibiotics alter the intestinal flora, suppressing the collaerogenes organisms and the clinical remissions seem to last until antibiotic resistant strains emerge to repopulate the gut. This event was apparently postponed in patients whose large intake of milk promoted the growth of lactobacilli.

"Untoward effects on salt and water balance have been observed in some cases treated with streptomyces fermentation products, and more experience will be needed before any satisfactory régime for their use can be evolved."

Lawrence
Long Island, N. Y

327 BARNARD R. D., KOPET S J., and STAHL, A. E. *Erythremia ("polycythemia") during massive, oral, Streptomyces-derived B_{12} therapy* Ann. Allergy 9 360-367 May June 1951.

Two cases are reported in which the patients received an "oral grade B_{12}" derived from the residue of an antibiotic-nonproducing Streptomyces strain. Both patients developed transient erythremia, but also experienced a marked abatement of allergic symptoms and a stabilization of a malignant leukoblastotic process. In one case the primary diagnosis was Hodgkin's lymphoma and in the other agnogenic myeloid metaplasia.

Queens, N. Y

328. WILSON S. J: *Observations on the effect of various folic acid antagonists on acute leukemia,* Blood 6 1002-1012, Nov 1951.

Prolonged but temporary remissions have occurred in patients with acute leukemia treated with folic acid antagonists. In the present study 70 patients were treated, 65 of whom survived for a sufficient length of time to evaluate the effect of the drugs. Folic acid antagonists used were aminopterin, a-methopterin, amino-an-fol, and a-ninopterin. Leukemias studied were acute lymphatic, monocytic and acute myelogenous leukemia. Eleven patients showed good response with remissions of 29 to 490 days. Of these 11 10 had lymphatic leukemia (8 of them were children under 10 years of age) Management of toxic reactions included vitamin B_{12} and liver extracts in most cases. Discontinuance of the antagonist at first signs of toxicity seemed the most effective procedure. Folic acid was not used

The summary reads, in part "When a response occurred, a rather definite hematologic pattern was noted. An increased platelet count in most instances was the first evidence of regeneration and occurred in about the third or fourth week. The neutrophilic polymorphonuclear leukocytes began to regenerate at about the same time and an increase in their number was followed by a rise in the erythrocyte count. Observations of the bone marrow indicated that although excellent clinical and hematologic remissions might occur primitive leukemic cells were still present. In some instances megaloblasts were observed in addition to a peripheral macrocytosis and anisocytosis of erythrocytes. Toxic manifestations were common. These included glossitis, ulceration of the oral cavity nausea, vomiting, diarrhea and alopecia. In one instance there was ulceration of the entire gastro-intestinal tract, including the esophagus and colon. Hematologic toxic reactions included thrombocytopenia, leukopenia and anemia. Aplasia of marrow tissue was observed in 1 instance. In many instances the margin of safety between a toxic reaction and death was indeed small."

University of Kansas School of Medicine
Kansas City Kan.

329 WELSH, I. *Failure of massive doses of vitamin B_{12} in acute leukaemia,* Brit. M. J 2 1133, Nov 22, 1952.

Twenty-five patients with acute leukemia (10 myeloblastic, 6 undifferentiated cell type, 5 lymphoblastic, 4 monocytic) were treated with daily 1-mg. doses of vitamin B_{12}. Therapy started at diagnosis and (in all but seven cases) continued until death. Transfusions and antibiotics were given as needed, but other remedies such as X-rays, cortisone or corticotropin were not given. None lived more than five months after diagnosis and beginning of treatment. There were no complete remissions. Six patients experienced partial remissions and 10 others reported feeling better The author states: " There is no evidence from this series to suggest that the prognosis or clinical course of acute leukaemia is altered by adding vitamin B_{12} to other treatment."

University of Witwatersrand
South Africa
Oxford, England

ANIMAL STUDIES

330. SKIPPER, H. E., BELL, M., and CHAPMAN J B. *Partial reversal of the antileukemic action of folic acid antagonists by nucleic acids,* Cancer 4 357 359 March 1951.

In a series of experiments in leukemic mice it was shown that desoxyribosenucleic acid and ribosenucleic acid will partially reverse the antileukemic action of folic acid antagonists. In a separate experiment it was noted that vitamin B_{12}, added to desoxyribosenucleic acid, provided an even greater reversal of an antagonist (amethopterin) than did DNA alone.

Southern Research Institute
Birmingham, Ala.

331 SKIPPER, H. E., CHAPMAN J B., and BELL, M. *Partial reversal of the anti-leukemic action of folic acid antagonists by vitamin B_{12},* Cancer Research 11 161-163, March 1951.

Eight separate experiments with controls were run on 228 mice injected with Ak 4 leukemia. Ninety mice were treated with the folic acid antagonists aminopterin or A-methopterin, and 138 were treated with the antagonists plus either B_{12} concentrate or crystalline B_{12}. Results showed that the addition of B_{12} partially reversed the anti-leukemic action of folic acid antagonists. With A methopterin, B_{12} shortened the life span an average of 2.6 days.

"It would appear that B_{12} in some obscure fashion is helping to provide chemical moieties (nucleic acid precursors) which are deficient in the leukemic cells of mice treated with folic acid antagonists. Such an interpretation is compatible with Shive's observation that B_{12} is involved in the biosynthesis of purines (or derivatives) as well as thymine in certain bacteria."

Southern Research Institute
Birmingham, Ala.

332. SKIPPER, H. E., CHAPMAN, J B., BOYD G A., RISER, W H., Jr., and BELL, M. *Preferential incorporation of formate carbon into leukemic blood cells as indicated by autoradiography* Proc. Soc. Exper Biol. & Med. 77 849-854, Aug. 1951.

One hour after injection of C^{14}-formate into mice with advanced leukemia, 90 per cent of the leukemic cells, in comparison to about 5 per cent of the more active lymphoid elements, are highly active. In the discussion, consideration is given to the possible part played by citrovorum factor coenzyme (thought to be involved in formate transfer) in normal and leukemic cells, by folic acid antagonists which inhibit the conversion of folic acid to citrovorum factor and inhibit incorporation of formate into nucleic acids, by folic acid deficiencies which inhibit incorporation of formate into proteins, by the antileukemic action of folic acid inhibitors, by the reversal of the antileukemic action of folic acid antagonists, by folic acid or citrovorum factor and by the partial reversal of this action by desoxyribose nucleic acid or vitamin B_{12}, and by other factors.

Oak Ridge Institute for Nuclear Studies
Oak Ridge, Tenn.
AEC Research Laboratories
Knoxville, Tenn.
Medical College of Alabama
University Ala.

Nervous System

NEUROLOGIC COMPLICATIONS

Subacute Combined Degeneration

333 UNGLEY C. C. *Anti-anaemic substances from liver* Lancet 1 771-772, May 15 1948 (In Letters to the Editor)

In the past six months, 3 pernicious anemia patients with subacute combined degeneration of the cord have been treated for periods of 21, 12, and 10 weeks with the highly purified material prepared from liver by the Glaxo Laboratories. In each case neurologic symptoms and signs have decreased progressively The degree of improvement has been in no way inferior to that observed in comparable cases in which crude liver extract has been administered.

334 BERK, L., DENNY-BROWN D., FINLAND M., and CASTLE, W B *Effectiveness of vitamin B_{12} in combined system disease rapid regression of neurologic manifestations and absence of allergic reactions in a patient sensitive to injectable liver extracts* New England J Med. 239 328-330, Aug. 26, 1948.

A patient with pernicious anemia and combined neurologic symptoms was treated with crystalline vitamin B_{12}. Hematologic remission and rapid improvement in the neurologic symptoms occurred after B_{12} therapy This patient was sensitive to liver extracts and had received folic acid therapy irregularly on previous occasions. The vitamin B_{12} was given intradermally (initial dose) and intramuscularly in a daily dose of 5 mcg. This amount is forty five times as great as that contained in 0.01 cc. of liver extract. On the ninth day of treatment the drug had to be stopped because of lack of supply The patient's reticulocyte and red cell count had increased and there was excellent improvement of the neurologic disorder A setback in neurologic improvement occurred when the drug was discontinued. After seven days, it was again administered, this time in doses of 5 mcg. intramuscularly three times a week, and steady improvement has resulted. Other than a small transient wheal at the site of intradermal injection, there were no allergic reactions attributable to vitamin B_{12}, which indicates that the B_{12} content is not responsible for sensitivity reactions caused by liver extracts.

Thorndike Memorial Laboratory and
Boston City Hospital
Boston, Mass.

335 SPIES, T D., STONE, R. E., GARCIA LOPEZ, G., MILANES, F., ARAMBURU T., and LOPEZ TOCA, R. *The association between gastric achlorhydria and subacute combined degeneration of the spinal cord*, Postgrad. Med. 4 89-95, Aug. 1948.

All of 160 adult patients suffering from macrocytic anemia (pernicious anemia, nutritional macrocytic anemia, macrocytic anemia of pregnancy tropical sprue) who were included in a study of the therapeutic effects of folic acid showed a hematologic response to this vitamin associated with remarkable clinical improvement. However in 28 of the 38 cases of pernicious anemia studied, subacute combined degeneration of the spinal cord developed, and progressed despite the folic acid, until liver extract was administered. Massive liver extract therapy gave prompt relief Each of these 28 patients had gastric achlorhydria prior to folic acid treatment. None of the patients with other types of macrocytic anemia had achlorhydria either before or during folic acid therapy and none developed subacute combined degeneration of the cord, which suggests a significant relationship between these two states.

Three patients suffering from addisonian pernicious anemia with achlorhydria and subacute combined degeneration, not included in the above series, were given 15 mcg. of vitamin B_{12} intramuscularly All showed prompt and remarkable subjective improvement. In the most acute case there was considerable improvement also in objective physical findings within two weeks, and in another case slight objective improvement was observed in a similar period.

Northwestern University
Chicago Ill.
University of Havana
Havana, Cuba

336. SPIES, T D., STONE, R. E., KARTUS, S., and ARAMBURU T *The treatment of subacute combined degeneration of the spinal cord with vitamin B_{12}*, South. M. J 41 1030-1031 Nov 1948.

In 3 patients with macrocytic hyperchromic anemia and subacute combined degeneration of the spinal cord, the neurologic status improved following the injection of vitamin B_{12}. One case is reported in detail—that of a 48 year old man whose neurologic condition was considerably improved after he had received four injections of 25 mcg. of vitamin B_{12}, given at intervals of 48 hours. His blood response also was good.

Northwestern University
Chicago, Ill.
Hillman Hospital
Birmingham, Ala

337 STONE, R. E., and SPIES, T D *Vitamin B_{12} and subacute combined degeneration of the spinal cord* Rev internat. de vitaminol. 20 228-233, 1948.

Three patients with pernicious anemia and subacute combined degeneration of the spinal cord who had been examined frequently over a long period were treated with vitamin B_{12}. In each instance the symptoms improved, and in 2 some of the abnormal physical findings were reversed. One of these cases is reported in detail. A 31 year old Negro woman was admitted to the Hillman Hospital in relapse a week after folic acid treatment had been discontinued. An intramuscular injection of 15 mcg of vitamin B_{12} was given. Her reticulocyte count rose from 2 to 13 per cent in seven days, fell to 2.2 on the thirteenth day and was 3.2 per cent on the sixteenth day when she was discharged. The initial blood counts of 2.66 million red cells and 3,400 white cells, and hemoglobin 65 per cent, increased to 3.39 million, 3,900, and 77 per cent, respectively Soreness of the tongue responded quickly neurologic symptoms improved, and strength in-

creased. When dismissed from the hospital the patient still complained of numbness of her feet and finger tips and slight stiffness of one knee. Five days later there had been no further improvement, and a second dose of vitamin B_{12} (9 mcg.) was given. Fourteen days later the blood values were 3.62 million red cells, 68 per cent hemoglobin, and 2.9 per cent reticulocytes, which were greater than had been obtained previously with folic acid or liver extract. Residual paresthesias persisted, but the patient gained weight and was able to be increasingly active.

338. HALL, B. E., and CAMPBELL, D. C.: *Vitamin B_{12} therapy in pernicious anemia. II Effect on the general clinical and neurologic manifestations preliminary report*, Proc. Staff Meet., Mayo Clin. 23 591-595, Dec. 8, 1948.

Clinical and neurologic responses to vitamin B_{12} in the same 11 patients with pernicious anemia in relapse whose hemopoietic responses were reported in Part I of this paper (Abstract 37) are presented here. The patients were observed for one to three and a half months. Improvement in strength, mental alertness and appetite, gain in weight, and disappearance of glossitis were noted. A patient with peripheral neuritis but without involvement of the spinal cord reported disappearance of paresthesias in one and a half months; 2 others noted improvement in one month, and further improvement may be expected. Five of 6 patients with peripheral neuritis and combined degeneration of the cord showed neurologic improvement, which was unusually rapid in 3.

339 JONES E.: *Vitamin B_{12}*, Am. Practitioner 3: 391 392, March 1949

The author briefly reviews the literature on vitamin B_{12} and states that his personal observations on 2 patients agree with the reports that vitamin B_{12} is of value in the treatment of combined system disease.

340. PATTERSON J F., STAUFFER, R., and FROMIN, A. M.: *The effect of vitamin B_{12} on hematologic and neurologic disorders in pernicious anemia report of a case*, Bull. New England M. Center 11 120-126, June 1949

341 METTIER, S R., McBRIDE, A., and TAT R. *The effect of vitamin B_{12} on the anemia and combined system disease of addisonian pernicious anemia*, California Med. 71 21 27 July 1949

Histories of 8 cases of pernicious anemia are presented. The 4 uncomplicated cases responded equally well to 50 or 25 mcg. of vitamin B_{12} given intramuscularly When the reticulocyte count had fallen, a secondary response was induced in only one of these patients by an injection of 25 mcg. indicating that the initial treatment had been suboptimum in this patient but optimum in the others. Improvement was maintained in the patients with injections of 25 mcg. of vitamin B_{12} every two weeks. The erythrocytes had returned to normal range within 60 days in the 3 patients who continued treatment. The fourth discontinued treatment and had a return of the anemia, which responded to renewed injections of B_{12}.

Two of the 3 patients who had pernicious anemia complicated by combined system disease showed an optimum, and the third a suboptimum, hemopoietic response to injections of vitamin B_{12}. Minor paresthesias and loss of vibratory sense disappeared in one of these patients and disturbances of a more serious degree in the others improved greatly

An eighth patient had been satisfactorily treated with liver extract until she became pregnant. She then became sensitive to liver extract, treatment was discontinued, and anemia recurred. Following the injection of 25 mcg. of vitamin B_{12} there was a satisfactory response of the blood, and there were no signs of drug sensitivity

The hemopoietic response in three patients was accompanied by change of the bone marrow from megaloblastic hyperplasia to normoblastic distribution. *Maintenance* injections of vitamin B_{12} may be from 30 to 50 mcg. at intervals of one month, depending on the individual case.

342. KRUSEN F H., HALL, B. E., and WOLTMAN H. W *Coordination exercises and vitamin B_{12} for combined degeneration of the spinal cord in pernicious anemia*, A.M.A. Scientific Assembly Official Program, June 1949 p. 195-196.

"If degeneration of the lateral and posterior columns of the spinal cord associated with pernicious anemia is diagnosed early and treated promptly decided improvement will often occur Treatment consisting of correct administration of vitamin B_{12} combined with proper instruction in coordination exercises has proved extremely effective. The results obtained in such management of 10 cases of combined degeneration of the spinal cord have been uniformly encouraging.

Authors' Abst.

343. HALL, B. E., KRUSEN F H., and WOLTMAN H. W *Vitamin B_{12} and coordination exercises for combined degeneration of the spinal cord in pernicious anemia*, J.A.M.A. 140 257-260, Sept. 24, 1949

The importance of coordination exercises in conjunction with vitamin B_{12} administration in patients with spinal cord degeneration associated with pernicious anemia should not be overlooked. Treatment with vitamin B_{12} and exercise must be started early before the axis-cylinders within the brain and cord have been destroyed, in order to obtain restoration of function.

The vitamin should be given in doses of 10 mcg intramuscularly each day or every second day for three to six months. Maintenance doses of 10 to 20 mcg. per week should follow the more intensive treatment. These doses are in excess of those needed by patients with pernicious anemia without involvement of the nervous system, and it is possible that even larger doses would be more effective. The exercises should be done during the period of vitamin B_{12} therapy

Twelve patients having subacute combined degeneration of the spinal cord associated with pernicious anemia were given combined parenteral administration of vitamin B_{12}, and coordination exercises. In three to nine months,

neurologic manifestations had disappeared or abated, in the majority of cases. Results are summarized in the accompanying table.

Neurologic symptom	No. cases	Recovered	Improved	Not improved
Paresthesia				
upper limbs	9	5	4	0
lower limbs	12	2	10	0
Ataxia	11	9	2	0
Romberg's sign	10	6	4	0
Vibration sense,				
lower limbs	12	1	7	4
Position sense, toes	10	4	5	1
Heel knee-toe test	11	8	3	0
Quadriceps femoris				
reflex	12	2	4	6
Triceps surae reflex	11	5	5	1
Babinski reflex	5	4	1	0
Clonus	1	1	0	0

344 MURRAY M. Y., and PROPP S.: *Vitamin B_{12} effective in pernicious anemia with combined system disease and liver sensitivity* New York State J Med. 49 2570-2572, Nov 1, 1949

Sensitivity to injected liver extract is not uncommon it was found in 17 per cent of 396 patients with pernicious anemia treated by Schwartz and Legere (*Blood* 1: 307 1946) In the case presented here, a patient with pernicious anemia developed severe sensitivity to liver extract, which was unrelieved by desensitization, antihistaminic drugs, and various other measures. Folic acid was substituted for the liver extract and although well tolerated failed to prevent glossitis and the progress of serious gastrointestinal symptoms. After about a year and a half of folic acid therapy signs of combined system disease appeared and advanced rapidly Folic acid was withdrawn, and 5 mcg. of vitamin B_{12} was given intradermally As no untoward reaction occurred, the patient was thereafter given 5 mcg. of this vitamin intramuscularly every day for six days, then 10 mcg. daily for nine days followed by 5 mcg. three times a week, and finally 10 mcg. once a week. Under this treatment a remarkable improvement took place in the combined system symptoms, the gastrointestinal symptoms disappeared, and the blood values returned to normal.

Albany Medical College
Albany N Y

345 UNGLEY C. C.: *Subacute combined degeneration of cord I Response to liver extracts. II Trials with vitamin B_{12}. Appendix quantitative method of assessing neurological status*, Brain 72 382- Sept. 1949 (abstr J.A.M.A. 143 399 May 27 1950)

The author has attempted to determine the nature of the agent in liver which is effective in subacute combined degeneration of the cord. Successful trials were made with a red crystalline anti-pernicious anemia factor vitamin B_{12}, one of the red pigments contained in Lester Smith's less pure earlier material. Eight patients with subacute combined degeneration were studied. The first 4 patients, who were given the less pure material initially have been treated for 14 to 19 months, for the past 13 of which they have received the pure substance. The remaining 4 patients treated for 9 to 13 months, were given only the crystalline material. The improvement after vitamin B_{12} therapy was compared with the "expected" improvement based on results obtained in 44 patients treated with liver extract. Four of the 8 patients exceeded the expected rate of improvement, 2 attained it and 2 fell just short of it. Vitamin B_{12} is therefore as effective as liver extract not only in pernicious anemia but also in subacute combined degeneration. Except in patients who are sensitive to liver extract, the author sees no advantage in using pure vitamin B_{12}. Weekly doses of 40 mcg of vitamin B_{12} or the equivalent amount in the form of liver extract is recommended for the first six months, followed by 20 mcg weekly as a maintenance dose thereafter

346. UNGLEY C. C.: *Liver extracts*, Lancet 1 708 1949

No significant difference was found between crude and "refined" liver extracts in treatment of 44 cases of subacute combined degeneration. Furthermore, vitamin B_{12} was as effective as liver extract, crude or refined, in this disease.

347 QUESTIONS AND COMMENTS: *Subacute combined degeneration*, Brit. M. J 2 1539 Dec. 31 1949

Treatment with liver extract or with vitamin B_{12} is recommended for a case of subacute combined degeneration of the cord with predominantly neurologic symptoms. With regard to vitamin B_{12}, it is stated that recent investigations have suggested its value in this condition but that as yet dosage and long-term effects have not been fully reported. It is suggested that doses of 40 to 60 mcg intramuscularly once or twice weekly be tried. A warning is given not to administer folic acid under any circumstances either alone or in combination with any other preparation. Administration of dilute hydrochloric acid and glycerin, pepsin, supplementary vitamins, especially B complex, and also ascorbic acid, a full normal diet, and massage and exercise are suggested as ancillary treatment. It is further stated that the response will vary according to the severity of the initial condition, the intensity and persistence of treatment, and the individual sensitivity of the patient, but that improvement should be noticeable in the first few weeks, particularly as the blood count returns to normal, after which it should be progressive although probably slow Paresthesias and some spasticity may persist for a long time.

348. JEQUIER, M., and HEMMELER, G *Treatment of pernicious anemia and funicular myelosis dangers of folic acid*, Praxis 39 63- Jan. 26, 1950 (abstr J.A.M.A. 143 1215, July 29 1950)

Five patients with pernicious anemia, who had responded favorably to liver therapy were given folic acid. All of the patients developed a neuroanemic syndrome after several months of treatment with folic acid. Folic acid was withdrawn and treatment was continued with liver extract and vitamin B_{12}. The folic acid did not exert any effect on glossitis which some of the patients presented. The authors observations warrant the conclusion that folic acid does not prevent the occurrence of funicular myelosis or its aggravation. Folic acid seems rather to induce involvement of the nervous system.

349 BERGER, H. *Vitamin B_{12} in addisonian pernicious anemia in a liver sensitive person*, New Y State J Med. 50 331-332, Feb. 1, 1950.

A woman with addisonian pernicious anemia developed sensitivity to liver. Folic acid controlled the hematologic situation, but symptoms of combined system disease developed. Treatment was changed to 10 mcg. of vitamin B_{12} once a week, and improvement in the neurologic symptoms was evident within five days. After six weeks of treatment there was no subjective or objective evidence of combined system disease. At this point the supply of vitamin B_{12} was exhausted, and it was necessary to substitute folic acid treatment. Neurologic symptoms returned under this treatment. Vitamin B_{12} is particularly valuable in that it apparently contains factors effective against both anemia and combined system sclerosis.

An addendum states that since these observations were originally made, Lubby (et al., abstr 180) has reported that vitamin B_{12} is not efficacious in the treatment of the megaloblastic anemias of infancy unless minute amounts of folic acid are present. This suggests that folic acid plays the role of a catalyst in this disease.

Richmond Memorial Hospital
Staten Island, N Y
Berger Clinic
Tottenville, N Y

350 LEDERER, M. *Deux cas de neuroanémie traités par la vitamine B_{12} (Two cases of neuroanemia treated with vitamin B_{12})* Semaine d. hôp. de Paris 26 478, Feb. 10 1950 (in Soc. Proc.)

In one woman, disturbances had developed progressively for four years, resulting in total helplessness, without a diagnosis having been made. Treatment with vitamin B_{12} led to significant improvement in the nerve lesions, and walking was possible at the end of four months. The blood picture improved spectacularly. The second woman had had pernicious anemia for 12 years. Neurologic disturbances which had been present for two years, in spite of folic acid treatment, disappeared rapidly under the influence of vitamin B_{12}.

351 MUELLER, J F., JARROLD T., HAWKINS V R., and VILTER, R. W *The effect of vitamin B_{12} on the hematologic and neurologic manifestations of pernicious anemia,* Ohio State M. J 46 225-229 March 1950.

In 7 patients vitamin B_{12} was found to be effective in the treatment of the hematologic, neurologic and glossal manifestations of pernicious anemia. Two cases are described in detail. A table is given which describes hematologic and clinical data on all of the patients.

It was found that 1 mcg. of vitamin B_{12} is roughly equivalent to 1 U.S.P unit of liver extract, and 12 mcg. usually induces a satisfactory hematologic remission. In patients with combined system disease 5 mcg. every other day induces a satisfactory clinical response. It is recommended that for routine treatment of pernicious anemia, 5 mcg. of vitamin B_{12} be given each day for 10 days and 5 mcg every week thereafter. If neurological abnormalities are found, at least 10 mcg. daily for several months is necessary.

It is concluded that there *is no evidence* that vitamin B_{12} has any more to offer a patient with pernicious anemia than liver extract. The one definite indication for vitamin B_{12} therapy in this disease at present is sensitivity to liver extract.

University of Cincinnati College of Medicine
Cincinnati, Ohio

352. GELIN G., and CANTÉGRIT M.: *Syndrome neuro-anémique biermérien datant de six ans traité par la vitamine B_{12} et la mécano thérapie (Biermer's neuro-anemic syndrome of six years duration treated with vitamin B_{12} and mechanotherapy)* Bull. Soc. méd. hôp. Paris 66 901-904, May 1950.

353. KIMMERER, J., and HUNTER, R. B.: *Therapeutic activity of vitamin B_{12} obtained from cultures of Streptomyc[es] griseus* Edinburgh M. J 57 2 1950 (abstr Schweiz. med. Wchnschr 80 662, June 24, 1950)

Results obtained with vitamin B_{12} (20 mcg. in 21 days) were similar to those obtained with liver and there were no neurologic or allergic side effects. Preexisting lesions in patients with funicular myelosis were not affected by higher doses (40 to 60 mcg.) of vitamin B_{12}.

354 FULD H. *Effect of vitamin B_{12} on neuropathy in pernicious anaemia treated with folic acid,* Brit. M. J 2 147 148, July 15, 1950.

A 74 year old pernicious anemia patient, after developing a sensitization dermatitis from concentrated liver injections, was given folic acid, 15 mg. daily. After 21 months of this therapy she was hospitalized and diagnosed as a case of severe psychoneuropathy following folic acid treatment. She also suffered from vitamin B deficiency. Treatment with a vitamin B_{12} preparation was begun. The initial dose was 2 cc. which is equivalent to 40 mcg. of vitamin B_{12}. It was then injected every third day for 15 days, every fifth day for 18 days longer and once a week for an additional two months. From then on she was kept on a maintenance dose of 2 cc. every two weeks. The patient had completely recovered within three months after the start of therapy

Broadgreen General Hospital
Liverpool, England

355 UNGLEY C. C.: *Vitamin B_{12} and other dietary factors in megaloblastic anaemias and in subacute combined degeneration of the cord,* Proc. R. Soc. Med. 43 537-541 July 1950.

356. NATARO, M. *The neurologic manifestations of pernicious anemia,* J Kentucky M. A. 48 360-365 Aug. 1950.

The literature on the neurologic manifestations of pernicious anemia is reviewed (29 references) and 6 cases are reported. Results of vitamin B_{12} therapy in these cases were similar to those obtained by adequate treatment with liver extract. Neurologic manifestations did not progress during treatment, but improvement could not be expected in all cases since most of them were of long duration. The author believes that the improvement in the neurologic manifestations under vitamin B_{12} treatment is the result of improvement in strength and general well-being and of correction of the anemia, as well as of specific effect. The part played by the lesions in the

spinal cord is debatable. The prognosis of the neurologic manifestations of pernicious anemia depends greatly on their severity and duration prior to treatment.

Louisville K

357 SUNDGREN V: *Developments in treatment of macrocytic hyperchromic anemias report of a case of pernicious anemia with sub-acute combined degeneration treated with vitamin B_{12}*, J Oklahoma M. A. 43 356-358, Aug. 1950.

The author presents a review of the development of specific methods of therapy in the hyperchromic macrocytic anemias, with particular reference to pernicious anemia (10 references) and describes a patient with pernicious anemia and subacute combined degeneration. The anemia responded to refined liver extract. After 24 days of liver therapy vitamin B_{12} became available and 10 mcg. daily was given. Thereupon the patient s neurologic signs regressed rapidly she has been maintained on 15 mcg. of vitamin B_{12} biweekly for a year and has shown no tendency to relapse. Of special interest is the fact that the patient's hair which was completely gray at the beginning of therapy has been gradually becoming darker It is concluded that vitamin B_{12} is a potent agent in the treatment of pernicious anemia, especially in cases with subacute combined degeneration.

Tulsa, Okla.

358. BORTZ, D W., and BATTLE, J D., Jr.: *Massive vitamin B_{12} therapy in pernicious anemia*, Cleveland Clin. Quart. 17 166-168, 1950 (abstr Mod. Med. 18 74 Sept. 15, 1950)

Massive doses of vitamin B_{12} improve the neurologic manifestations of pernicious anemia more rapidly and completely than the usual small amounts. A woman who was unable to walk alone because of severe progressive posterolateral sclerosis was out of bed for several hours a week after intramuscular injection of 1,000 mcg. of vitamin B_{12}, and in 13 weeks she could walk without a cane, although numbness, tingling, and burning sensations continued. Walking ability was further improved by a second large dose, although vibratory perception did not change.

359 QUERIES AND MINOR NOTES *Subacute combined degeneration of spinal cord*, J.A.M.A. 144 1224-1225 Dec. 2, 1950

A doctor s condition has been diagnosed as lateral posterior sclerosis of the spinal cord, and the writer asks about the cause, prognosis and treatment of the condition. The editor replies that the condition is usually associated with pernicious anemia but infrequently is also seen in secondary anemia and severe dietary inadequacies. If recognized early in the course and before spinal cord changes are severe, neurologic manifestations are frequently reversible. Treatment should be directed at the underlying or associated disease. In the treatment of pernicious anemia, the administration of liver extracts and/or vitamin B_{12} will protect against the development or effect remission of subacute combined degeneration of the spinal cord. Folic acid is not effective. The provision of vitamin B complex as a part of the therapeutic regimen may control symptoms of peripheral neuritis, which frequently accompanies this disease.

360. DOIG R. K., MOTTERDAM R., ROBERTSON E. G., and WOOD I J: *Early diagnosis of subacute combined degeneration of the cord Value of gastric biopsy* Lancet 2 836-841, Dec. 23 1950

The authors discuss the difficulties of early diagnosis of subacute combined degeneration of the cord. They emphasize the value of gastric biopsy in determining the presence of atrophy of the gastric mucosa.

Five cases are described in detail. In one, treatment with vitamin B_{12} was begun (results not given) In another patient, gastric biopsy showed only minimal atrophy The patient was therefore considered a case of alcoholic peripheral neuritis and not one of pernicious anemia with subacute combined degeneration of the cord. She responded to a diet high in calories and vitamins plus parenteral vitamin B complex with considerable improvement in her mental and physical state.

Walter and Eliza Hall Institute of Medical Research, and
Royal Melbourne Hospital
Melbourne, Australia

361 UNGLEY C. C., and CAMPBELL, H.: *Effects of vitamin B_{12} in pernicious anaemia and subacute combined degeneration of the cord*, Brit. M. J 1 152-157 Jan. 27 1951.

Authors summary "The therapeutic value of vitamin B_{12a}, in pernicious anaemia was assessed and compared with that of vitamin B_{12}.

"Twenty-eight responses to single doses were observed in 24 patients. Doses were graded logarithmically —10, 20, 40, 80, and 160 μg. The increase of red blood cells in 15 days after a given dose showed the usual individual variations, but the mean of 28 responses was almost identical with the response expected from equal doses of vitamin B_{12}. The log. dose–response curve did not differ significantly from that of vitamin B_{12}. The greater consistency in response to doses of 40 μg. and over suggests that such doses would be more useful than smaller doses in future therapeutic trials.

"Twenty patients with little or no neurological involvement have been maintained for four to nine months on doses of 10 μg. every two weeks. Red blood cell or haemoglobin values were temporarily slightly subnormal in two patients: in one normality was restored with iron, and in the other without alteration of treatment. A third patient had a slightly raised M.C.V [mean corpuscular volume] None of the 20 patients showed neurological, lingual, alimentary or other symptoms of relapse. These results are as good, so far as those observed with similar maintenance doses of vitamin B_{12}. Such low maintenance would be dangerous, however in patients not under close observation. For routine purposes we normally give at least 60 μg. every three weeks—more if there is neurological involvement or intercurrent infection.

"The efficacy of vitamin B_{12a} against the nervous disorders of pernicious anaemia was tested in six patients with subacute combined degeneration of the cord, two whom were subjected to quantitative studies, and in patients with minor neurological involvement. N

the patients became worse and all except two were improved.

"This experiment has failed to show any difference between vitamins B_{12a} and B_{12}, but even a difference of 80% in haemopoietic potency would result in only a difference of about 5% in the average response of 28 cases: a large difference in dosage causes only a small increase in response. Later analyses based on increases of hemoglobin and of packed cell volume in 15 days also failed to demonstrate any difference in potency between the two vitamins. Although similar limitations apply to these results they greatly strengthen the conclusions based on increase of red blood cells alone. Both vitamins are effective against the neurological manifestations of the disease."

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

362. SCHEINBERG P: *Cerebral blood flow and metabolism in pernicious anemia*, Blood 6 213-227 March 1951

The author has measured cerebral blood flow and metabolism in 16 patients with pernicious anemia. In 7 of the patients additional studies were made during various stages of therapy Seven of the patients received vitamin B_{12} for periods ranging from 1 to 18 months. One patient received folic acid and liver extract and the remaining patients received liver extract or no treatment.

At the time of study the patients could be divided into two equal groups those with severe anemia and those with moderate or no anemia, the latter groups having shown hematopoietic response to treatment. In the first group, cerebral blood flow was increased and cerebral vascular resistance decreased, in the second group, cerebral blood flow was decreased and vascular resistance increased. In both groups, cerebral oxygen and glucose consumption was decreased, as was cerebral venous oxygen tension. A good correlation was found between the mental status defects and cerebral oxygen consumption and between severity of neurologic involvement and cerebral oxygen consumption. There was no correlation between cerebrovascular resistance and cerebral oxygen consumption, nor between degree of anemia and cerebral oxygen consumption.

Specific therapy with liver extract or B_{12} produced slight to great neurologic improvement in patients with neurologic symptoms. It also produced a moderate increase in cerebral oxygen consumption and cerebrovascular resistance. In no instance did cerebral oxygen consumption become normal.

The author discusses the disparity between the functional ability of the patients and the low values for cerebral metabolism. They conclude that pernicious anemia results in specific nervous system involvement not related to the anemia, and that this damage is at least partially irreversible in many patients.

Duke University School of Medicine
Durham, N C.

363. SLEPIAN A., and VAUGHN, S. L. *Nervous system manifestations in pernicious anemia Results of treatment with liver preparations as compared with B_{12} therapy* New York State J Med. 51 1524-1526, June 15, 1951.

On initial examination of 36 patients with pernicious anemia, 28 (78%) had neurologic abnormalities varying from peripheral neuropathy to signs and symptoms of posterolateral tract disease. Treatment was intensive both initially and in maintenance therapy; over a 21 year period it has consisted of oral liver first, then oral liver extracts, and finally parenteral extracts. One patient, in addition to these 28, after several months of treatment with folic acid alone developed spinal cord degeneration. With treatment, in the patients who had only peripheral neuritis the condition was reversed; in those without neural symptoms spinal cord disease did not develop. Those with cord involvement showed progression in their lesions, but adaptability to the neurologic disease improved in all, and under the long maintenance treatment the improvements were retained. The more extensive the disease the greater was the tendency for neurologic progression. Those faithful in their maintenance therapy progressed in their disease just as much as those with suboptimal therapy The results suggest that prompt, vigorous treatment of neurologic and blood relapses may achieve almost the same end point. A study of these patients and others under the effects of B_{12} will be reported later

University of Buffalo School of Medicine, and
Buffalo General Hospital
Buffalo, N Y

364 CONLEY C. L., and KREVANS, J R.: *Development of neurologic manifestations of pernicious anemia during multivitamin therapy* New England J Med. 245 529-531 Oct. 4, 1951.

Five patients are described who had neurologic symptoms characteristic of subacute combined degeneration, but whose blood showed little or no anemia. All 5 patients had taken large amounts of multivitamin preparations for long periods. It is known that the administration of folic acid can overcome the blood abnormalities in pernicious anemia, while allowing the neurologic disorder to progress. It was possible to identify the multivitamin preparation used by 4 of the 5 patients, and in each case the folic acid content was adequate to produce a hematologic remission in patients with pernicious anemia. All 5 patients were treated with intramuscular vitamin B_{12} and in all the neurologic symptoms disappeared.

Since folic acid deficiency is quite rare, it does not seem necessary or prudent to routinely employ multivitamin preparations to which folic acid has been added. However since these multivitamin mixtures are in widespread use, physicians must be especially careful to watch for the early manifestations of pernicious anemia. The demonstration of histamine-refractory achlorhydria in a patient with this neurologic disorder is an indication for intensive therapy with vitamin B_{12}, even though the blood and bone marrow are normal. The incorporation of small amounts of vitamin B_{12} in multivitamin preparations does not protect patients with pernicious anemia against the development of subacute combined degeneration. Vitamin B_{12} is poorly absorbed from the gastrointestinal tract in patients with this disease, and the vitamin must be given parenterally for optimal effect.

Johns Hopkins University and Hospital
Baltimore, Md.

365 CHODOS R. B., and ROSS, J F: *The effects of combined folic acid and liver extract therapy* Blood 6 1213-1233, Dec. 1951

It has been found that folic acid therapy sometimes exerts a deleterious effect on the central nervous system of patients, especially those with pernicious anemia. The present study concerns patients with pernicious anemia treated initially with folic acid and later with a combination of folic acid and vitamin B_{12} or liver extract.

Authors summary "Folic acid, when administered alone, did not prevent the development or progression of subacute combined degeneration in 12 of 22 patients receiving this agent for from twelve to twenty five months. One patient with total gastrectomy and a macrocytic anemia developed subacute combined degeneration after five months of folic acid therapy Folic acid therapy did not produce neurologic disease in patients with iron deficiency anemia who had free gastric hydrochloric acid in their gastric secretions and presumably sufficient intrinsic factor It did not influence response to ferrous sulfate therapy Neurologic disease did not develop in 6 pernicious anemia patients treated with folic acid and liver extract for three and one-half to thirty-nine months. In 10 pernicious anemia patients with good nutrition, neurologic relapses did not progress when liver extract or vitamin B_{12} therapy was instituted, even though folic acid therapy was continued. In 2 patients with abnormal nutrition and complicating organic abnormalities, nervous system disease progressed after institution of liver extract therapy The hematologic status of patients with pernicious anemia is not maintained in a more satisfactory state by supplementation of liver extract or vitamin B_{12} therapy with folic acid."

These findings are explained by the theory "that the hematologic and neurologic manifestations of pernicious anemia and other macrocytic anemias associated with gastro-intestinal tract pathology and inadequate nutrition are due to a deficiency of more than one substance. The administration of folic acid may improve the hematologic status but induce a deficiency of another substance or substances, e.g., vitamin B_{12}, which are essential for the maintenance of a normal blood picture and the integrity of the central nervous system. This deficiency will eventually result in the development of a suboptimal blood picture or subacute combined degeneration of the spinal cord, or both."

The authors recommend that patients with sprue, nutritional macrocytic anemia and other macrocytic anemias associated with gastrointestinal tract pathology who are treated with folic acid should also be given supplemental liver extract or vitamin B_{12} to insure against the development of nervous system disease.

Massachusetts Memorial Hospitals, and
Boston University School of Medicine
Boston, Mass.
Cushing VA Hospital
Framingham, Mass.

366. SAMSON D C., SWISHER, S. N., CHRISTIAN R. M., and ENGEL, G L.: *Cerebral metabolic disturbance and delirium in pernicious anemia: clinical and electroencephalographic studies* A.M.A. Arch. Int. Med. 90 4-14, July 1952.

In 13 of 14 patients with pernicious anemia in hematologic relapse, tests showed a reduction in awareness justifying the diagnosis of delirium. This was presumed to be the result of a disturbance in cerebral metabolism and was accompanied with more or less irreversible electroencephalographic changes. Under treatment with vitamin B_{12}, the delirium regressed and the electroencephalogram began to show return toward normal at about the time when the reticulocyte response began, which suggests that the cerebral defect is primary rather than secondary to the anemia. The mental improvement was primarily on the level of awareness, and not on the more personal aspects of behavior A patient with paranoid trends improved in awareness but showed no change in his paranoid behavior The authors state that there is no reason, from their studies, to anticipate that vitamin B_{12} or related antianemia factors will affect other psychiatric disorders or even delirium in patients who do not have pernicious anemia.

Strong Memorial Hospital,
Rochester Municipal Hospital, and
University of Rochester School of Medicine and Dentistry
Rochester N Y

367 BASTRUP MADSEN P: *Cytology of bone marrow in non-anemic funicular myelopathy (subacute combined degeneration of spinal cord)* Ugesk. f. laeger 114 1853- Dec. 25, 1952 (abstr J.A.M.A. 152 288, May 16, 1953)

"Bastrup-Madsen presents further clinical evidence that the spinal cord lesions of pernicious anemia can occur as an independent neurological affection that leads to disability or continues for years without the development of a regular pernicious anemia. Ten cases of subacute combined degeneration of the spinal cord with histamine-fast achylia are described. In six cases the hemoglobin and erythrocyte values were normal, and in four cases there was a slight reduction in the erythrocyte count. Examinations of the bone marrow revealed slight maturation disturbances in the erythropoiesis in seven cases In all cases there were perniciosiform disturbances in maturation of granulocytes, testifying to a lack of antipernicious anemia factor as the basis for the myelopathy In five cases examinations of the bone marrow after two weeks of antipernicious anemia treatment showed normal bone marrow In two additional cases there was a slight rise in the erythrocytes during treatment. In five cases some improvement in the neurological symptoms occurred during the specific treatment. In nine cases the disorder had persisted for from 2 to 20 years, and in seven cases more or less disability had developed before the start of antipernicious anemia treatment. In these cases specific antipernicious anemia treatment must be instituted without waiting for the development of anemia. Examination of the bone marrow may be a valuable diagnostic aid even though the peripheral blood shows normal conditions. The same substances, liver and stomach preparations, and vitamin B_{12}, seem to be important for both the central nervous system and the bone marrow"

368. EARL, C. J., EL HAWARY M. F S., THOMPSON R. H. S., and WEBSTER, C. R.: *Blood pyruvate levels in subacute combined degeneration of cord effect of vitamin B_{12} therapy* Lancet 1: 115-11 Jan. 17 1953.

Raised blood pyruvate levels were found in 3 patients with untreated subacute combined degeneration of the cord. Parenteral treatment with 140 to 200 mcg. of vitamin B_{12} per week in divided doses led to a rapid return to normal of these levels.

The neurologic symptoms and blood counts improved in all 3 patients, but this occurred more slowly than did the normalization of pyruvate blood levels.

The authors come to the tentative conclusion that "in subacute combined degeneration of the cord there is a defect of pyruvate metabolism which can be rectified by treatment with vitamin B_{12} "

Guy's Hospital Medical School London, England

369 HOWARD R., in discussion on Hammarsten, J F *Clinicopathological Conference, Minneapolis Veterans Hospital, Presentation of case*, Journal Lancet 73 101 102, 118, March 1953.

Dr Howard reports having seen a patient with pernicious anemia who had an exacerbation of associated neurologic complications after taking a mail-order preparation containing B_{12} and folic acid.

University of Minnesota Minneapolis, Minn.

Diabetes Mellitus

370. SCOTT R. W., SANCETTA, S. M., and AYRES, P R.: *A preliminary note on the use of vitamin B_{12} in the management of the neurologic manifestations of diabetes mellitus*, Tr A. Am. Physicians 63 232-240, 1950.

371 SANCETTA, S. M., AYRES, P R., and SCOTT R. W *The use of vitamin B_{12} in the management of the neurologic manifestations of diabetes mellitus, with notes on the administration of massive doses*, Ann. Int. Med. 35 1028-1048, Nov 1951.

Since vitamin B_{12} has been found beneficial in the neurologic changes in patients with pernicious anemia, the authors have used it, together with insulin and dietary control, in 12 diabetic patients with neurologic disturbances. Case histories are given. Three patients are regarded as having complete neurologic remission, 1 had a partial relapse during treatment, followed by complete remission, 3 are considered in almost complete remission, 2 as improved, and 2 as questionably improved. The response in such patients varies in accordance with the extent of vascular damage and the reversibility of injury to nerve cells. Damage to nerve tissue as a result of arteriosclerosis of the vasa nervorum is presumably irreversible. It is doubtful that intrinsic spinal cord lesions can be reversed. Improvement is not to be expected unless effective regulation of diabetes with insulin and diet is instituted.

Exact dosage of vitamin B_{12} has not been established. A flexible dosage of 15 to 30 mcg. daily for the first 7 to 14 days is recommended, followed by a maintenance dosage of 15 to 30 mcg. once or twice a week. Frequency of administration appears to be more important than the amount of individual doses in achieving and maintaining a neurologic remission. Crystalline vitamin B_{12}, given intramuscularly or subcutaneously was used except in one patient who was treated exclusively with streptomycin broth concentrate B_{12}.

Concentrations of 900 and 1,000 mcg. of vitamin B_{12} per cc. were used in some patients. In one of them the administration of 900 mcg gave results similar to those obtained with 15 mcg. Two whose residual symptoms consisted of occasional pain in the feet at night were not relieved by daily injections of 900 mcg. each for five days. One patient, an alcoholic who showed no improvement until satisfactory control of the blood sugar had been attained, was refractory to small doses of vitamin B_{12} but responded dramatically to 1,000 mcg. doses 10 such doses were given on alternate days. A second course was given later About a month after vitamin B_{12} had been discontinued, three doses of 1,000 mcg. each were given at weekly intervals, followed by 15 mcg. once a week, which keeps the patient free from pain except for dull aches occurring on the sixth day after each injection. It is doubtful if the enormous doses used in this case were necessary The authors conclude that massive dosage of vitamin B_{12} may be indicated in the exceptional patient showing severe manifestations, but it is doubtful that improvement can be attained more quickly and completely than with the usual small doses. The improvement of these patients appears to be the result of the action of vitamin B_{12} on the deranged intrinsic nerve metabolism, insofar as the latter is not partially caused by obliterative disease of the vasa nervorum. The mode of action is not known.

Western Reserve University School of Medicine Cleveland, Ohio

372. BEIDLEMAN B. *Clinical vitamin deficiencies in patients with diabetes mellitus*, J Clin. Nutrition 1 119-123, Jan. 1953.

Patients with diabetes are often given diets low in calories or fat which do not supply enough vitamins. Perifollicular hyperkeratosis and dry skin may result from vitamin A deficiency Theoretically vitamin D deficiency might result from these diets, but the author has not encountered signs of it in diabetics.

Peripheral neuritis, neurasthenia, anorexia, burning sensations in the mouth, soreness and redness of the tongue, cheilitis, tenderness along the major peripheral nerve trunks, and absence of tendo Achilles reflexes are the most frequently encountered signs of vitamin B deficiencies. Thiamine alone may clear the signs and symptoms of peripheral neuritis, and lesions of the skin and mucous membranes may respond to thiamine, riboflavin, or niacin, or even pyridoxine or biotin. Although the major deficiency may be of a single vitamin, deficiencies of the others may account for some of the symptoms. Thiamine with riboflavin and niacin has been suggested for the treatment of diabetic coma, but the author has not found that omission or addition of large doses of this vitamin complex influences the rate of recovery from coma.

Ascorbic acid and rutin have been suggested for treatment and prevention of diabetic retinopathy because of their effect on the integrity of the capillary wall the author is prescribing three tablets a day each containing 100 mg. of ascorbic acid and 20 mg. of rutin, but has seen no reversal of retinopathy although its progression may have been slowed.

In patients whose diabetes has not been well controlled hepatic enlargement which responds to choline may occur

True diabetic neuropathy is manifested by signs and symptoms similar to those seen in patients with the combined system disease of pernicious anemia. Many patients with mild to moderate neuropathic symptoms have been promptly and lastingly relieved by daily administration of 30 mcg. of vitamin B_{12} for three to five days. Some patients require a maintenance dose of this vitamin to prevent recurrences. In 1 patient ataxia, nocturnal diarrhea, peripheral neuritic symptoms, and a left sixth cranial nerve palsy completely disappeared within a month of vitamin B_{12} therapy In some patients severe neuropathies appear to have reached an irreversible stage, and vitamin B_{12} has only a slight effect or none at all even in large doses over prolonged periods.

Pennsylvania Hospital, and
Jefferson Medical College
Philadelphia, Pa.
(now at)
Medical Center Clinic
Pensacola, Fla.

373. MENOF P *New treatment for polyneuritis*, South African M. J 24 715- Aug. 26, 1950 (abstr J.A.M.A. 144 1521 Dec. 23, 1950)

Vitamin B_{12} was administered to patients with alcoholic and diabetic polyneuritis. The doses used were considerably larger than those recommended for pernicious anemia. The author began with 60 mcg. [and finally gave as much as 210 mcg.] in one injection. The best result was achieved with this large dose. The treatment was given to 11 patients with encouraging results. Apart from a little localized tenderness due to the large size of the intramuscular injection (7 cc.) no undesirable side effects were noted. The author feels that vitamin B_{12} might be effective in other forms of polyneuritis and in other nervous disorders associated with alcoholism, e.g., delirium tremens, Korsakoff's psychosis, hemorrhagic polioencephalitis, and alcoholic hallucinosis. Vitamin B_{12} might also be tried in such diseases as progressive muscular atrophy

374 MENOF P: *Role of vitamin B_{12} in polyneuritis fatty degeneration and pernicious anaemia*, South African M. J 25 894-899 June 9 1951 (abstr J.A.M.A. 147 791, Oct. 20 1951)

"Menof recently called attention to the remarkable curative action of vitamin B_{12} in the polyneuritides associated with chronic alcoholism and diabetes mellitus. He reported a dramatic response in nine of 11 cases. Since that time he has treated 12 similar patients (seven alcoholic, four diabetic, and one diabetic and alcoholic) The response has been uniformly satisfactory The tenderness and volume of the liver receded almost as rapidly as the neurological disturbances. The author feels that vitamin B_{12} should be used wherever fatty degeneration is encountered. It is also indicated in any condition where demyelination or defective red blood cell structure is believed to be the underlying pathological factor Reasons are given why pernicious (Addison's) anemia should now be regarded as a disorder of lipoid metabolism rather than as a disease of the blood. Evidence is adduced to show that the action of vitamin B_{12} is to synthesize the various chemical substances that make up certain phosphatides or cerebrosides or both."

Neuropathies in General

375 BEAN W B., FRANKLIN M., and SACHS A. L.: *Preliminary note on the effect of vitamin B_{12} on the painful aspects of nutritional neuropathy* Proc. Central Soc. Clin. Research 22 10-11, Nov 1949 (*also* abstr Mod. Med. 18 108, Feb. 1 1950)

Because it has been questioned whether vitamin B_1 actually is the factor concerned in the neuropathies of beriberi, and because vitamin B_{12} has successfully controlled combined system disease in pernicious anemia, the authors tried vitamin B_{12} in 14 patients with painful neurologic disorders of other origins. In 2 patients the neurologic symptoms were associated with nutritional deficiency and in 1 with chronic alcoholism and diabetes. In 11 they apparently were unrelated to nutritional factors. The first three patients had dramatic relief of pain following one intramuscular injection of 15 mcg of vitamin B_{12}. In the remaining 11 patients, who were suffering variously from brachial neuritis, multiple sclerosis, diabetic neuritis, tabes, trigeminal neuralgia, monocytic leukemia, ruptured disk and alcoholic psychosis, no relief of pain was obtained by this treatment.

376. BEAN W B., FRANKLIN, M., and SACHS A. L. *An effect of vitamin B_{12} on pain in nutritional neuropathy* Am. J M. Sc. 220 431-434, Oct. 1950 (*also* cited in Nutr Rev 9 207-208, July 1951)

The authors describe 3 patients with painful nutritional neuropathy in whom the pain disappeared within 30 to 60 minutes after the injection of 15 mcg. of vitamin B_{12} concentrate. Adequate control studies were made. The patients subsequently showed improvement on a good diet supplemented with yeast, vitamin B complex tablets, riboflavin and niacin.

Twenty five patients with pain caused by a miscellaneous group of neurologic disturbances did not respond to the administration of vitamin B_{12} concentrate.

The mechanism of action of B_{12} is unknown. Its effect, however is believed to be direct. Moreover an experiment is cited which shows that the results obtained are not an effect of analgesia.

In an addendum, a case is cited in which the patient improved after the injection of 6 mcg. but not of 3 mcg. of vitamin B_{12}. Another patient who was moribund did not respond to 80 mcg of B_{12}.

State University of Iowa College of Medicine, and
University Hospitals
Iowa City Iowa

377 SPILLANE, J D *Vitamin deficiencies and the nervous system*, Practitioner 2 143- 1950 (abstr Schweiz. med. Wchnschr 80 926, Aug 26, 1950)

In animal experiments, deficiencies of vitamins A and E and B complex factors lead to neurologic disturbances. In man, only the members of the B complex are of importance to the nervous system. Thiamine is necessary for cellular respiration, and riboflavin, nicotinic acid, pyridoxine and pantothenic acid are necessary for proper functioning of the nervous system. It is pointed out that polyneuritic symptoms frequently occur in patients with gastrointestinal diseases and in alcoholics whose nutrition is inadequate. It is possible that spinal cord degeneration is caused by vitamin B_{12} deficiency. This condition is often associated with pernicious anemia and it improves when the anemia improves as a result of vitamin B_{12} therapy.

378. HARVIER, P., CLAISSE, R., and CARAMANIAN M. K. *Syndrome neuroanémique grave non biermerien. Guerison spectaculaire par la vitamin B_{12} (Spectacular cure with vitamin B_{12} of a severe neuroanemic syndrome—not Biermer's anemia)* Semaine d. hôp. de Paris 26 3757 Sept. 25, 1950 (In Soc. Proc.)

A severe neuroanemic syndrome occurring in the course of serious posthemorrhagic anemia (not pernicious) was almost completely cured by eight days' treatment with vitamin B_{12}. The ataxic and bedridden patient recovered his normal aspect and activity within a few days.

NEUROLOGIC DISEASE

Amyotrophic Lateral Sclerosis

379 SPIES T D., and STONE, R. E.: *Some observations on patients with amyotrophic lateral sclerosis* South. M. J 42: 410-411, May 1949

Five cases of progressive muscular atrophy due to spinal cord degeneration were selected for treatment with vitamin B_{12}. Four of these were diagnosed as amyotrophic lateral sclerosis. In the fifth, the symptoms were similar except that no involvement of the lateral column was evident. These patients had been affected for one to four years, and many kinds of treatment had failed. In all 5, the disease was progressive. In each case there was great subjective improvement, with diminution in muscle cramps and stiffness, following the administration of vitamin B_{12}. While there were no measurable objective changes, there was no progression of the disease process during several weeks of observation, and treatment will be continued to see whether the disease process can be halted by frequent injections of the vitamin. The patient whose case is summarized in this article was given 10 mcg. of B_{12} three times a week.

Birmingham, Ala.

380. SPIES, T D., HAUSER, E., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., ARAMBURU T., and STONE, R. E.: *Observations sur l'action de la vitamine B_{12} sur des sujets atteints sclé-roses combinées, associées a l'anémie pernicieuse la sclérose en plaque la sclérose laterale amyotrophique et les séquelles de la poliomyelite (Observations on the effect of vitamin B_{12} on patients with combined system disease associated with pernicious anemia, multiple sclerosis, amyotrophic lateral sclerosis, and residual changes of poliomyelitis)* Internat. Ztschr f. Vitaminforsch. 21 347-358, 1949

The administration of adequate doses of vitamin B_{12} ameliorated the symptoms of combined sclerosis, especially the acute symptoms. In all but 1 of the 60 cases with pernicious anemia and associated combined system disease the clinical and hematologic response to vitamin B_{12} was good. This one patient relapsed while under treatment: thiamine was ineffective but liver extracts brought about a response. In multiple sclerosis, amyotrophic lateral sclerosis and residual changes of poliomyelitis, B_{12} brought about some subjective but little objective improvement. The administration of 15 mcg. of vitamin B_{12} five times a day in 40 cases of acute poliomyelitis gave no amelioration except a slight subjective amelioration in 4 cases. The authors consider B_{12} the most effective substance at present available for use in Biermer's macrocytic, and nutritional anemias, and in sprue.

Multiple Sclerosis

381 LEREBOULLET J., PLUVINAGE, R., and COTY R. *Vitamine B_{12} et sclerose en plaques (Vitamin B_{12} and multiple sclerosis)*, Presse méd. 58 230, March 4, 1950 (In Soc. Proc.) J.A.M.A. 143: 1272, Aug. 5, 1950 (In Foreign Letters)

Seven patients with multiple sclerosis characterized by extreme sensitivity in addition to the usual signs of this disease were treated with large doses of vitamin B_{12}. The sensitivity disappeared and there was great functional amelioration. A more prolonged study is needed to determine whether the pyramidal pain is likewise affected. In discussion, Perault emphasized the interest of this communication which may contribute to the explanation of the mechanism of action of vitamin B_{12} on the neurologic-anemic syndrome. Significant amelioration of neurologic signs is often observed before the hematologic disturbance is completely ameliorated.

382. JONEZ, H. D: *Certain vascular effects of histamine and d-tubocurarine in multiple sclerosis. Part III* Ann. Allergy 8 188-193, March-April, 1950.

In a series of 254 patients treated by intravenous injections of histamine, many received adjunctive treatment with d-tubocurarine and vitamin B_{12}.

"The allergy theory of multiple sclerosis etiology and therapy does not conflict with or displace the therapy calling for the prevention of vasospasm and continued vasodilatation."

3[illegible] *reatment of multiple*

a revie , J.A.M.A. 143

Aug.

In a review article on the drugs which have been used in the treatment of multiple sclerosis, the author cites an article of his own, soon to be published, on the treatment of multiple sclerosis with vitamin B_{12}.

384 SIMSON G., HERFORT A., KRIM, M., and MEYER, L. M : *Effects of vitamin B_{12} in multiple sclerosis*, Proc. Soc. Exper Biol. & Med. 75: 721 Dec. 1950.

Five patients with multiple sclerosis and 1 patient with amyotrophic lateral sclerosis failed to respond clinically or hematologically to daily intramuscular injections of 1,050 mcg. of vitamin B_{12}. Treatment was continued for three weeks in a case of multiple sclerosis of one month's duration the other patients, who had all been ill for years, were treated for four months (1 case) or five months.

Goldwater Memorial Hospital
Welfare Island, N Y

385. BOOTH, C. B., LAWYER, T., Jr., and von STORCH, T J C. *Vitamin B_{12} in the treatment of multiple sclerosis*, J.A.M.A. 147 894, Oct. 27 1951 (in Correspondence)

Because of conflicting reports in the literature regarding the effects of vitamin B_{12} in multiple sclerosis, the writers made a study of 24 patients with a justifiable diagnosis of multiple sclerosis who were treated with vitamin B_{12}. Six of the patients were treated in a hospital and 18 as outpatients. Six patients received 100 mcg. of vitamin B_{12} intramuscularly every other day for three months, and 5 of these received 1 000 mcg. of the vitamin every other day for an additional three months. Fifteen patients received 100 mcg. of vitamin B_{12} three times a week for three months, 2 received 1,000 mcg. of vitamin B_{12} three times a week for three months, and 3 have received 1,000 mcg. of the vitamin three times a week for two months, and therapy is continuing.

About one-fourth of the patients noted an increase in appetite and a sense of well being while receiving vitamin B_{12} therapy There was, however no objective evidence of neurologic improvement aside from the type of fluctuation which is characteristic of the natural course of the disease. After cessation of therapy two patients claimed that they felt much stronger and were definitely benefited by vitamin B_{12}. They were therefore given placebos for two months, and reported the same dramatic improvement with the placebo as they claimed for vitamin B_{12}. It is concluded that vitamin B_{12} is not effective in the treatment of multiple sclerosis.

Montefiore Hospital
New York, N Y

386. LEREBOULLET J., and PLUVINAGE, R. *Use of vitamin B_{12} in neurology* J.A.M.A. 148 667 Feb. 23, 1952 (in Foreign Letters)

" Drs. J Lereboullet and R. Pluvinage reported on the use of vitamin B_{12} in the treatment of 74 patients with neurological disease. In 10 of 43 patients with multiple sclerosis, there were no positive reactions in 16 patients with advanced cases, there were 4 instances of only subjective improvement. In 17 patients with cases of average intensity there was noticeable improvement and, in 4 instances, the patients were able to resume their normal occupations after years of illness and failure of all previous treatment. In eight patients with spastic paraplegia, including two cases of unknown origin and two cases that occurred after serious accidents, functional improvement was observed. In six out of eight patients with spinocerebellar syndromes of the acromegaly type, functional improvement and a diminution of ataxia were noted four patients, formerly invalids, were able to resume their former activities. In two children with cerebellar atrophy vitamin B_{12} brought about an ambulatory state and a considerable diminution of objective cerebellar signs. In seven patients with polyneuritis, including three with Korsakoff's psychosis, results were spectacular the three patients with Korsakoff psychosis were cured within one week. The authors believe that vitamin B_{12} has a positive action on early polyneuritis but has no effect on old lesions. In regard to the mechanism of action of vitamin B_{12}, the authors consider two hypotheses. The first is that vitamin B_{12} acts on myelinic elements, especially in neuroanemic syndromes and in certain cases of multiple sclerosis, in which demyelination, with comparative conservation of axons, occurs. The second hypothesis assumes an occasional direct stimulation of functions of the elements not destroyed."

Herpes Zoster

387 JOLLES, K. E. *Herpes zoster treated with vitamin B_{12}*, Brit. M. J 1 959 April 22, 1950 (In Correspondence)

Six cases of herpes zoster were treated with vitamin B_{12} concentrate with uniformly excellent results. The stages of the disease at which therapy was instituted varied from early intense erythema before vesiculation to a case of heavily secondarily infected herpes zoster with considerable underlying cellulitis. The treatment has not yet been tried in cases of postherpetic neuralgia.

Vitamin B_{12} was given in intramuscular injections of 2 cc. of the concentrate on the first, second, and fourth days of treatment. Injection was absolutely painless. Considerable clinical and objective improvement followed the first injection, new lesions ceased to appear and by the end of the first week all local signs of the disease were gone.

West Bromwich, England

388. HOWARD C. *Liver extract for herpetic pain*, Lancet 2 242, Aug. 2, 1952.

The writer describes 4 patients with herpes to substantiate his claim that liver extract helps relieve herpetic pain

"In 3 of them the pain disappeared in twenty four hours, never to return. In the 4th the pain, which was agonising and involved the area supplied by the *left* brachial plexus, disappeared, but was succeeded by intense itching. In each case 'Campolon 2 ml. was *injected* intramuscularly and it seemed to me that the change of infliction in the last case might be due to an insufficient amount of some constituent in the liver extract.

"I therefore quite empirically gave an injection vitamin B_{12} ('Cytamen 2 ml.) and the itching van

overnight. When I last saw the patient a week ago he had been free from symptoms, except slight numbness, for a month."

London, England

389 LEITCH, G B. *Vitamin B_{12} in massive dosage for herpetic lesions a preliminary report*, Northwest Med. 52 291 292, April 1953.

Because of favorable reports of the effect of vitamin B_{12} on neurologic conditions in pernicious anemia and on trigeminal neuralgia, the author administered it to a patient for severe pain from herpes zoster which had resisted various types of treatment. The vitamin was used in the strength employed in treating trigeminal neuralgia —1,000 mcg. in a volume of 10 cc., half of which was injected into each buttock. This was repeated on the next day by the third day a preparation containing 1,000 mcg. per cc., was available and was substituted for the less concentrated solution. Herpetic pain disappeared within 24 hours after administration of the first injection, and the lesions began to heal within 48 hours. After five days the dosage was reduced to 500 mcg. on alternate days. After the second 500 mcg. injection the patient left the hospital one more 500 mcg. dose and two of 100 mcg. each, on alternate days, concluded the treatment. The usual peripheral neuritis was so mild that the patient refused treatment. In 4 other cases, which were less severe, vitamin B_{12} was equally successful. In only one of these cases was mild sedation for peripheral neuritis required. In a single case of herpes simplex involving the lips, the patient was able to preach after three injections of 1,000 mcg. each although crusts remained. There were no unfavorable reactions to daily injections of 1,000 mcg. of vitamin B_{12}.

The author considers further trial of vitamin B_{12}, alone or with other remedies such as thiamine hydrochloride, indicated in herpes. The only reference which he found in the literature was a report by Heyblon (*Méd. français* 11 102, May 1951) of recovery from herpes zoster in two to six days in 8 of 11 patients treated with vitamin B_{12} the recommended dosage was 30 mcg. daily for eight days.

Portland, Oregon

Other Neurologic Disorders

390. ARKLESS, H. A. *Reactions to vitamin B_{12}*, J.A.M.A. 144 1586, Dec. 30, 1950 (in Correspondence)

Subcutaneous injections of vitamin B_{12} concentrate were given to a 47 year old woman who had multiple radicular neuralgias of long duration. The first four injections were without reaction the fifth and sixth were followed by swelling and pain in both arms. No general effects were noted by the patient the swelling subsequently disappeared.

325 So. Sansom St.
Philadelphia, Pa.

391 JAMIESON S. R.: *Tuberculous meningitis treatment with streptomycin and para-aminosalicylic acid*, Brit. M. J 1 83-85, Jan. 12, 1952.

In addition to intramuscular and intrathecal streptomycin combined with oral PAS all patients showing muscular wasting were given one tablet of a proprietary vitamin B_{12} preparation daily These patients increased in weight, had improved appetites and became more active.

Castle Hill Hospital
Cottingham, East Yorkshire
England

392. FIELDS W S., and HOFF H. E. *Relief of pain in trigeminal neuralgia by crystalline vitamin B_{12}*, Neurology 2 131 1952.

Thirteen patients with trigeminal neuralgia experienced remarkable relief after treatment with large intramuscular doses of vitamin B_{12}. Six patients received 1,000 mcg. of vitamin B_{12} biweekly or triweekly for four to eight weeks. The lancinating pain gave way to a burning paresthesia which disappeared later Supplies of the drug were not available for 2 of the patients for about one week shortly after the typical attacks stopped. In both, the lancinating pain returned but was relieved after the first of six daily 1,000 mcg. injections. The last 7 patients were treated with 10 daily doses of 1,000 mcg. of vitamin B_{12}.

Nine of the patients had not had previous surgical intervention, and these experienced prompt and complete remissions. Relief from pain was delayed in the 4 cases where surgery or nerve block had been resorted to, but "ultimately results appeared to be equally [as] good" as for the other 9 patients.

Baylor University College of Medicine
Houston, Texas

393 PHILLIPS, G B., VICTOR, M., ADAMS, R. D., and DAVIDSON, C. S. *A study of the nutritional defect in Wernicke's syndrome the effect of a purified diet, thiamine, and other vitamins on the clinical manifestations*, J Clin. Investigation 31 859-871 Oct. 1952.

Authors summary and conclusions "1. Nine patients with Wernicke's syndrome, characterized by ophthalmoplegia, nystagmus, ataxia, and mental disturbances were given a purified diet composed solely of glucose and minerals. Specific vitamins were added after appropriate intervals of observation.

"2. Prior to the administration of thiamine, there was no improvement in any of the signs. More specifically despite alcohol withdrawal, bed rest, and the addition of other vitamins (niacin, calcium pantothenate, *pyridoxine, folic acid, ascorbic acid, riboflavin, B_{12}*), the ophthalmoplegia progressed and the ataxia remained unchanged, while the nystagmus decreased only in association with an increase in ocular paralysis.

"3. When thiamine only was added to the purified diet, the ophthalmoplegia cleared considerably in from one and a quarter to six hours diminution in nystagmus and ataxia also occurred in some of the patients, but the change was more gradual there was improvement in mental disturbance, but it was minimal in degree and consisted of increased attentiveness and capacity to maintain a conversation and greater ease of confabulation.

"In view of these observations, there seems to be little doubt that the ophthalmoplegia of Wernicke's syndrome is related to a specific lack of thiamine. The nystagmus and ataxia also appear to be related to thiamine deficiency but the evidence is less conclusive. No definite conclusions can be drawn regarding the relationship of the mental disturbances to the deprivation of thiamine or other vitamins."

Thorndike Memorial Laboratory
Massachusetts General Hospital,
Boston City Hospital, and
Harvard Medical School
Boston, Mass.

394 FRIEDLANDER, W J: *Meralgia paresthetica* U S Armed Forces M. J 3 1857-1862, Dec. 1952.

Meralgia paresthetica occurring in 14 soldiers is described. The disease, which is a neuropathy of the lateral femoral cutaneous nerve, is not uncommon, but is often misdiagnosed. Three of the patients were given 120 mcg. of vitamin B_{12} intramuscularly daily for two weeks, but this treatment had no beneficial effect. The disease usually disappears spontaneously but sometimes nerve resection or procaine nerve block is necessary

U S Army Hospital
Fort Bragg N C.

ANIMAL STUDIES

395. HOGAN A. G., O'DELL, B. L., and WHITLEY J R. *Maternal nutrition and hydrocephalus in newborn rats*, Proc. Soc. Exper Biol. & Med. 74 293-296, June 1950.

The incidence of hydrocephalus in the young of female rats given an experimental diet containing soybean oil meal and a vitamin mixture that included all recognized vitamins except ascorbic acid, niacin, folic acid and B_{12} was less than 1 per cent. When folic acid deficiency was produced by adding a folic acid antagonist, crude methyl-folic acid, to the diet, the incidence of hydrocephalus rose to 20 per cent. There was a long delay in the appearance of hydrocephalic young when the females were kept on a casein diet before transfer to the soybean oil meal diet containing a folic acid antagonist. The casein diet did not contain folic acid, and the delay in appearance of hydrocephalus may indicate that another nutrient is involved, possibly vitamin B_{12} since the stock diet undoubtedly contains it and the casein used contained 1.8 mcg. per 100 cc., while soybean oil meal is known to be a poor source.

University of Missouri
Columbia, Mo.

396. O'DELL, B. L., WHITLEY J R., and HOGAN A. G.: *Vitamin B_{12}, a factor in prevention of hydrocephalus in infant rats*, Proc. Soc. Exper Biol. & Med. 76 349-353, Feb. 1951

Rats that had been maintained from weaning to maturity on a diet deficient in vitamin B_{12} were then given the same diet with supplements of a folic acid antagonist. Twenty-three per cent of their young were hydrocephalic and only 17 per cent were weaned at the age of 4 weeks. Rats given the same experimental diet without previous depletion produced 15 per cent hydrocephalic young and weaned 21 per cent; rats without previous depletion that were given a vitamin B_{12} concentrate (animal protein factor supplement) which supplied 2.2 mcg of vitamin B_{12} per 100 Gm. of diet produced no hydrocephalic young and weaned 81 per cent.

Rats that had consistently produced hydrocephalic young were given 1 mcg. of crystalline vitamin B_{12} each week parenterally No hydrocephalic young were produced by rats in which vitamin B_{12} administration had been started five weeks before parturition or on the seventh day of gestation, but in the latter group only 14 per cent of the young were weaned. When vitamin B_{12} was not given until the sixteenth day of gestation, 50 per cent of the young were hydrocephalic.

Supplements of folic acid alone did not prevent hydrocephalus in the young. The soybean oil meal used in the basal diet in these experiments contains some vitamin B_{12} and it is not certain whether vitamin B_{12} alone is effective or whether both folic acid and vitamin B_{12} are required.

University of Missouri
Columbia, Mo.

397 ALEXANDER, W F., and BACKLAR, B. *Nucleic acid changes in rat nerve tissue after parenteral administration of vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 78 181-184, Oct. 1951.

Authors summary "These preliminary results support the contention that vit. B_{12} is related to the genesis of ribonucleoproteins in rat tissues. Weanling and immature rats were placed in 2 dietary groups, chow and basal, the latter dietary group deficient in vit. B_{12}, folic acid and PABA. One-half of each group received parenteral B_{12} every day Liver spinal cord and cervical sympathetic ganglia were examined histologically for changes in pentose and desoxypentose nucleic acids. These 3 tissues from animals which received vit. B_{12} showed more DNA than the controls. No consistent observable difference was seen in spinal cord and cervical sympathetic ganglia for the DNA as visualized by the Feulgen stain. There was a slight increase in the intensity of the Feulgen stain in hepatic cell nuclei from rats which had received vit. B_{12} over the controls."

St. Louis University School of Medicine
St. Louis, Mo.

Sprue

398. SPIES, T D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and CULVER, B.: *Observations on the hemopoietic response of persons with tropical sprue to vitamin B_{12}*, South. M. J 41 523-525, June 1948.

Two patients with tropical sprue in relapse were each given a single intramuscular injection of 8 mcg. crystalline vitamin B_{12}. Each responded clinically and hematologically By the third day the patients voluntarily said that they felt stronger and by the sixth day soreness of the mouth and tongue had completely disappeared, appetite had returned, and the stools had decreased in volume. Both patients showed a rise in reticulocytes which reached a peak on the eighth day This was followed by an increase in red blood cells and hemoglobin and, in 1 case, by a rise in white blood cells.

Northwestern University
Chicago, Ill.
Gen. Calixto Garcia Hospital
Havana, Cuba

399 SUAREZ, R. M., and SPIES, T D: *The effectiveness of vitamin B_{12} in the treatment of tropical sprue*, Bol. Asoc. med. de Puerto Rico 40: 199-209 Aug. 1948.

400. SPIES, T D., and SUAREZ, R. M.: *Response of tropical sprue to vitamin B_{12}*, Blood 3 1213-1220, Nov 1948.

The response of 5 patients with tropical sprue to vitamin B_{12} given intramuscularly is described. Two patients received three single doses of 10 to 25 mcg. at various time intervals, 1 patient received two single doses of 25 mcg., 1 a single dose of 20 mcg. and 1 a single dose of 4 mcg. An injection of 10 to 25 mcg. always produced a reticulocytosis, beginning usually about the fourth day This was followed by erythrocytosis and hemoglobin production. Striking clinical improvement, evidenced by increase in strength and vigor and decrease in diarrhea, coincided with reticulocytosis. No patient showed a maximum hematologic response and each tended to relapse quickly both clinically and hematologically but another injection of vitamin B_{12} was promptly effective. A single injection of about 100 mcg. probably is necessary to produce the maximal hematologic response in patients as seriously ill as those studied. The patient who received 20 mcg. of vitamin B_{12} showed spectacular improvement 15 days later following ingestion of 400 Gm. of liver and responded 30 days later to 20 mcg. of pteroyltriglutamic acid per day intramuscularly The patient given 4 mcg. of vitamin B_{12} showed little or no hematologic response, but 100 mcg. of pteroyldiglutamic acid per day orally produced a satisfactory response. The 2 patients who received three single injections of 10 to 25 mcg. of vitamin B_{12} have shown steady clinical and hematologic improvement.

Northwestern University
Chicago, Ill.
School of Tropical Medicine
Puerto Rico

401. SPIES, T D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and CULVER, B.: *The hematologic and clinical response of persons with tropical sprue to vitamin B_{12}*, Rev internat. de vitaminol. 20 209-216, 1948.

Reports are presented of 4 cases of tropical sprue in Cuba which were treated with vitamin B_{12}. Three patients received 15 micrograms each [apparently as a single dose], and the fourth 23 micrograms. The number of reticulocytes began to rise on the third to fifth day after treatment and reached a peak of 13.2–26.1 per cent on the seventh or eighth day falling later to 4.4–7.2 per cent. The red blood cell count rose from initial values of 1.41–2.50 million to 2.12–2.70 million on the final day; the corresponding white blood cell counts were 2,900–10,150 rising to 6,350–14,150, and the hemoglobin values, 38–57 rising to 45–60 per cent.

Clinical improvement began a day or two after vitamin B_{12} was administered. Soreness and redness of the tongue lessened and, after a few days, disappeared. Appetite and strength increased, the number of stools decreased and their character improved. One patient was discharged at his own request on the tenth day the others were observed for twelve days.

402. ADLERSBERG D: *Newer advances in sprue*, Oral Surg., Oral Med., & Oral Path. 1: 1109-1118, Dec. 1948.

The treatment of sprue is discussed in a general article. In addition to dietary therapy liver extract or folic acid and vitamin concentrates are given. Crystalline vitamin B_{12} has a remarkable hemopoietic activity and its role in the treatment of sprue is being studied.

Beth Israel Hospital, and
Mount Sinai Hospital
New York, N Y

403 SUAREZ, R M., SPIES, T D., HERNANDEZ-MORALES, F., and PEREZ, E.: *A note on the effectiveness of vitamin B_{12} in the treatment of tropical sprue*, Blood 4 1124-1130, Oct. 1949

Three patients with tropical sprue were treated over prolonged periods with intramuscular injections of vitamin B_{12} in order to determine whether full remission might be obtained by giving much larger amounts of the vitamin than are indicated by the dramatic hemopoietic response to minute doses. One patient received a total of 210 micrograms, in nine injections of 10 to 25 micrograms, in a period of 147 days another 205 micrograms in nine injections of 10 to 25 micrograms in 160 days and the third, 200 micrograms in eight 25 microgram injections in 138 days.

None of the patients showed any change for the first three or four days. They began to feel better however on the fourth and fifth day, with the beginning of reticulocyte rise. After the reticulocyte peak had been reached (on the sixth to ninth day) the red blood cells and hemoglobin gradually increased. Each patient slowly gained strength, and the two who had diarrhea showed some improvement in alimentary tract function although the stools did not become entirely normal. Therapeutic responses

have been sustained. It is stated that no agent thus far used in the treatment of tropical sprue has been so effective per unit of weight as vitamin B_{12}.

404 RODRIGUEA-MOLINA, R., RAMIREZ-RODRIGUEZ, E. A., ACEVADO C. E., LOPEZ, U., TORRES, J M., and OLIVELLA, J A.: *Oral administration of vitamin B_{12} concentrate in tropical sprue and nutritional macrocytic anemia*, Acta Haematologica 6 277-283, Nov 1951 (abstr Quart. Rev Med. 9: 103-104 May 1952)

Authors abstract "Vitamin B_{12} concentrate, in amounts varying from 25 to 300 µg daily orally administered over periods of 5 to 7 days to two cases of acute sprue and to one case of nutritional macrocytic anemia, produced no significant hematopoietic response, and questionable clinical improvement. One case of chronic sprue presented slight subjective improvement but questionable hematologic response. When 150 and 300 µg. daily were administered to a case of pernicious anemia in relapse, a rapid sustained hematologic and clinical improvement was observed. However no improvement in the long standing neurologic status was noted."

San Juan, Puerto Rico

405. SUÁREZ, R. *News and views Foreign newsletter—Puerto Rico* Blood 6 1293-1296, Dec. 1951.

The incidence and treatment of sprue in Puerto Rico are described. A report concerning megaloblastic anemia of pregnancy included one case responding to vitamin B_{12}. Sprue is an ever-present problem. Good results have been obtained with oral folic acid (2.5 mg. daily) vitamin B_{12} (15 mcg weekly by injection) citrovorum factor and a proprietary mixture of ferrous sulfate, vitamin B_{12}, folic acid, and ascorbic acid. Results were poor or absent when the following were tried ACTH 20 mg. every six hours for 14 days, euphoria but no clinical improvement 500 mg. and 100 mg. oral single doses of vitamin B_{12} combined folic acid and vitamin B_{12}. A patient with mild symptoms of sprue developed a fulminating degeneration of the posterolateral cord while receiving pteroyldiglutamic acid.

Puerto Rico

406. DIÉZ RIVAS, F., HERNANDEZ MORALES F., and MEYER, L. M. *The oral use of combined vitamin B_{12} and folic acid in tropical sprue*, Ann. Int. Med. 36 1076-1085, April 1952.

In 6 cases of tropical sprue in relapse the daily administration of 1.67 mg. of folic acid and 25 mcg. of vitamin B_{12} in tablet form brought about satisfactory hematologic and clinical remission. The results were comparable to those obtained with parenteral liver therapy When folic acid is given alone the minimal effective oral dose is 10 to 15 mg. a day and that of vitamin B_{12} is 150 to 200 mcg., but when the two are given simultaneously smaller doses are effective. It appears that the macrocytic anemia of tropical sprue and pernicious anemia is a multiple deficiency state and that both folic acid and vitamin B_{12} are needed for its correction. The synergistic action of folic acid and vitamin B_{12} suggests that folic acid is needed for the proper absorption and utilization of vitamin B_{12} by the human organism. The extrinsic factor described by Castle may be a combination of substances among which folic acid and vitamin B_{12} play a prominent role.

University of Puerto Rico School of Medicine
San Juan, Puerto Rico
Kings County Hospital
Brooklyn, N Y

407 DIEZ RIVAS, F., SUAREZ, R. M., HERNANDEZ MORALES F., and PEREZ SANTIAGO E. *The oral administration of vitamin B_{12} in tropical sprue* Ann. Int. Med. 36 583-591 Feb. 1952 (Part 2)

The chemistry and source of vitamin B_{12} are discussed briefly together with parenteral dosage, clinical use of the vitamin and its oral administration in pernicious anemia. Studies in 10 patients with active sprue and definite macrocytic anemias indicate that the oral use of vitamin B_{12} is not the most efficient or practical way to treat the disease. Small single doses of vitamin B_{12} (15 to 50 mcg.) by mouth effected a hematopoietic response in 6 of 8 cases (optimal in 1 and suboptimal in 5) and failed in 2. Large single doses (90 to 450 mcg.) failed to induce a response in 3 out of 4 cases. Large multiple daily doses (100 to 400 mcg.) in 2 cases produced a suboptimal response in each case definite clinical improvement occurred in 6 weeks in one case and in 12 weeks in the other

The minimal oral dose of vitamin B_{12} which will produce an appreciable reticulocytosis and definite clinical improvement when given orally appears to lie between 150 and 200 mcg. daily for two to three weeks or longer Folic acid, 15 mg. orally daily or 15 mcg. of vitamin B_{12} intramuscularly is more effective than vitamin B_{12} orally either with or without gastric juice. Although simultaneous administration of 75 to 150 cc. of gastric juice increased the effect of oral vitamin B_{12} in 2 of 3 cases, the potentiating effect was less than has been observed in the treatment of pernicious anemia by Castle or at the Mayo Clinic, although larger doses were used. The authors state "This difference may have been due to the difference in the source of the vitamin B_{12}. In this study the Squibb's preparation was used in eight cases, while Castle and Hall and Campbell used vitamin B_{12} supplied by Merck laboratories." Another explanation offered is that in sprue the intestinal absorption of the extrinsic factors or of the antipernicious anemia factor (vitamin B_{12}) might be impaired.

University of Puerto Rico School of Tropical Medicine
San Juan, Puerto Rico

408. SUAREZ, R. M., SABATER, J., SUAREZ, R. M., JR., and BUSO R.: *The effect of sublingual administration of crystalline vitamin B_{12} in tropical sprue*, Blood 7 749-754 July 1952.

Authors summary: "Sublingual administration of crystalline vitamin B_{12} in daily doses of 25 µg. for ten consecutive days failed to induce clinical or hematologic remissions in 5 patients with tropical sprue."

Hospital Mimiya
Santurce, Puerto Rico

IDIOPATHIC STEATORRHEA

409 TUCK, I. M., and WHITTAKER, N *Vitamin B_{12} in idiopathic steatorrhoea*, Lancet 1 757 759 April 22, 1950

The authors describe 2 cases of megaloblastic anemia associated with idiopathic steatorrhea one of them was first diagnosed post partum (neither had had any evidence of diarrhea) Neither case responded to adequate intramuscular doses of vitamin B_{12} which had been proved fully active in Addison s anemia in the dosage given to the present cases (20 to 40 mcg.) Both recovered promptly on therapy with folic acid (20 to 30 mg. by mouth daily for 25 days)

The authors review the work of the other investigators on the use of vitamin B_{12} in several types of anemia. They state that apparently cases of megaloblastic anemia of pregnancy and refractory cases of megaloblastic anemia with free hydrochloric acid have not been generally investigated for the presence of idiopathic steatorrhea.

These two cases emphasize the importance of looking for a defect in fat absorption in cases of megaloblastic anemia refractory to liver or vitamin B_{12}, even in the absence of characteristic bowel symptoms.

North Middlesex Hospital England

410. ISRAELS M. C. G., and SHARP J : *Idiopathic steatorrhea (nontropical sprue) with megaloblastic anemia*, Lancet 1 752-757 April 22, 1950

The authors describe in detail 5 cases of idiopathic steatorrhea (nontropical sprue) with megaloblastic anemia, and discuss the diagnostic difficulties—particularly the fact that the fat absorption defect may be missed. Parenteral liver treatment and vitamin B_{12} in a dose sufficient to produce a full remission in pernicious anemia yielded no response in these cases. All of the patients responded promptly to folic acid, given usually by mouth in a 20- or 10-mg. dose. One patient responded satisfactorily to the synthetic conjugate of folic acid, pteroyltriglutamic acid, in oral doses of 120 mg. daily In several of the cases folic acid was given intravenously also, in a dose of 100 or 150 mg. So far blood counts have been maintained by this treatment. In the cases in which they were present, folic acid relieved infantilism and amenorrhea without supplementary endocrine treatment. It is concluded that the megaloblastic anemia of nontropical sprue is one of the group of liver-resistant folic-acid responsive anemias which includes most of the megaloblastic anemias, with the exception of addisonian pernicious anemia.

University of Manchester and Manchester Royal Infirmary Manchester England

411 COOKE, W T., PEENEY A. L. F., and HAWKINS, C. F : *Vitamin B , in idiopathic steatorrhoea*, Lancet 1 834, April 29 1950 (in Letters to the Editor)

The writers discuss the fat absorption defect in idiopathic steatorrhea, and state that in their first 100 cases, less than half had symptoms of intestinal disturbance. A number of the remaining patients had macrocytic anemia which was distinguishable from pernicious anemia only by virtue of a fat absorption defect and the failure to obtain normal blood values with adequate liver extract therapy In the writers experience folic acid invariably produces favorable results in megaloblastic anemias associated with idiopathic steatorrhea. Three cases are reported which showed a maximum reticulocyte response after parenteral vitamin B_{12}.

University of Birmingham Birmingham, Ala.

412. ZUMOFF B. *Possible relationship of folic acid to uric acid metabolism as exemplified by a case of nontropical sprue*, Am. J M. Sc. 225 674-676, June 1953.

A case of nontropical sprue with megaloblastic anemia was found to have hyperuricemia during relapse. The blood uric acid level decreased toward normal after the anemia was successfully treated with folic acid.

The first diagnosis had been pernicious anemia, and both vitamin B_{12} and liver extract were given, without clinical improvement.

Theoretical and experimental evidence was cited to support the hypothesis that hyperuricemia may sometimes be a chemical sign of folic acid deficiency

Jewish Hospital of Brooklyn Brooklyn, N Y

OTHER GASTROINTESTINAL CONDITIONS

413 SNORF L. D *Chronic ulcerative colitis*, M. Clin. North America: 243-255, Jan. 1951 (Chicago Edition)

Treatment of chronic ulcerative colitis includes papaverine hydrochloride, up to one grain three to six times a day to control intestinal unrest, vitamin B_{12} parenterally and antibiotics. Antibiotics are indicated where there is evidence of systemic infection penicillin, in doses of 300,000 to 600,000 units for ten days, has been found to give temporary improvement. Dihydrostreptomycin and aureomycin have been found ineffective, except that aureomycin is of benefit when intestinal involvement extends beyond the bowel wall. Sulfonamides have been generally ineffective, though it is thought they control secondary invasion of the bowel wall by intraluminal bacteria.

Northwestern University Medical School Chicago, Ill.
Evanston Hospital Association Evanston, Ill.

414 RAIL, G A. *Two cases of ulcerative colitis treated with vitamin B_{12}*, Lancet 2: 816-817 Nov 3, 1951 (abstr J.A.M.A. 148 582, Feb. 16, 1952)

Because diets high in liver have been instrumental in healing residual ulcers in cases of amebiasis, and on the premise that ulcerative colitis might be caused by a dietary deficiency, 2 cases of ulcerative colitis were treated with vitamin B_{12}. Both cases were diagnosed by exclusion, both were presumed to be of about five years standing, and neither patient had ever been treated for ulcerative colitis.

The first patient, a 31-year-old woman, received 20 mcg of vitamin B_{12} daily for one week. She was then discharged from the hospital feeling well and having no diarrhea. Injections of B_{12} were given twice weekly for almost three months. Sigmoidoscopy a month after the start of treatment showed complete healing of the bowel. More than a year later symptoms had not recurred.

In the second patient, a 52 year-old man, vitamin B_{12} therapy was less intensive. A preliminary five-day course of penicillin plus sulfadiazine cleared up a mass in the left iliac fossa. Thereafter the patient received vitamin B_{12} plus oral Casydrol (60 minims three times a day) He was discharged from the hospital after four daily injections of 20 mcg. of B_{12}. One month and six injections later the patient had an excellent appetite and no diarrhea. Subsequent treatment was intermittent, and after an additional two and one-half weeks course of B_{12} the patient refused further therapy Some five months after admission sigmoidoscopy showed the bowel to be completely normal. No relapse had occurred to the time of writing, 18 months after the start of treatment.

In summary the author states "Both cases had, at different times before their illness, been on a diet inadequate, in many ways, when compared with that to which they were accustomed."

415 CHEWNING C. C., Jr. *Colitis following the oral administration of aureomycin and terramycin*, Virginia M. Monthly 79 136-137 March 1952.

The author has recently noted colitis in patients who have been treated with oral aureomycin or terramycin. The colitis was characterized by diarrhea, abdominal pain, edema and erythema of the rectal and sigmoidal mucosa, and in severe cases ulceration of the mucosa. The pathogenesis of this colitis is not understood. Candida albicans was not found in the 2 patients whose stools were examined for this organism. The author refers to a personal communication regarding use of vitamin B_{12} during oral administration of aureomycin; this measure was believed helpful in preventing complications.

Richmond, Va.

416. HIRSON C. [*Diabetic nocturnal diarrhea*] Lancet 2 1227 Dec. 29 1951 (In Letters to the Editor)

The writer describes a case of diabetic nocturnal diarrhea of four months duration in which symptoms were controlled by full doses of vitamin B_{12}, improvement having lasted for two months to the time of writing.

New End Hospital
Hemel Hempstead, Herts., England

417 QUERIES AND MINOR NOTES *Perianal irritation due to aureomycin*, J.A.M.A. 150 627 Oct. 11, 1952.

A physician requests information on side effects of aureomycin. He has a patient who developed severe perianal irritation after taking 10 capsules of 250 mg. each of aureomycin for a respiratory infection. The editor answers that the irritation in and around the perianal tissues caused by aureomycin and terramycin is dependent on an interference with the normal flora of the colon, the resulting changes in enzyme balance seeming to produce the irritation. Dr A. Miltzer of Michael Reese Hospital in Chicago has observed that if these patients are given two teaspoonfuls of an elixir of vitamin B_{12}, folic acid, and ferric ammonium citrate four times a day with the aureomycin or terramycin, neither diarrhea nor perianal irritation occurs. Dr Miltzer believes that aureomycin and terramycin destroy the normal flora of the intestinal tract, particularly those organisms which are involved in the synthesis of vitamin B_{12}. It is also pointed out that there is an overgrowth of saprophytes and particularly Pseudomonas in patients receiving aureomycin and terramycin, and these organisms contribute to the diarrhea.

418. CONWAY N S., and CONWAY, H. *Vitamin B_{12} and folic acid in megaloblastic anaemia after total gastrectomy* Brit. M. J 1 158-161 Jan. 27 1951

The authors report what they believe is the first recorded case of megaloblastic anemia following total gastrectomy to have been treated with vitamin B_{12}. The B_{12} was injected in the form of the concentrate (prepared from Streptomyces griseus) the initial dose being 40 mcg.) Striking clinical improvement followed vitamin B_{12} therapy but the hematologic response was suboptimal compared with that expected in addisonian pernicious anemia. After 280 mcg. of vitamin B_{12} concentrate had been given over a period of almost two months, folic acid (20 mg. daily) was given in addition. A complete hematologic remission followed. The authors remark on the fact that the "dumping syndrome" cleared with the improvement in the patient's general condition. They discuss the pathogenesis of the anemia in the light of the classic theory of erythropoiesis. They believe that this type of megaloblastic anemia and addisonian pernicious anemia are not identical although they are similar morphologically They discuss the relationship of the anemia described to other megaloblastic anemias with respect to treatment with vitamin B_{12} and folic acid. Thirty four references are cited.

University of Glasgow, and
Western University
Glasgow, Scotland

419 SWENDSEID M. E., HALSTED J A., and LIBBY R. L. *Excretion of Cobalt[60]-labeled vitamin B_{12} after total gastrectomy* Proc. Soc. Exper Biol. & Med. 83 226-228, June 1953.

Authors summary: "Co^{60}-labeled vitamin B_{12} was administered orally in 0.5 γ doses to 4 patients who had had a total gastrectomy Each patient excreted in the stools essentially all of the administered radioactivity When gastric juice was given with the vitamin there was a decrease in Co^{60} excretion to levels similar to those found in control subjects."

Veterans Administration Center, and
University of California Medical Center
Los Angeles, Calif.

420. BERMAN J K., HABEGGER, E. D., and BILLINGS E.: *Massive resection of the small intestine a six-year follow-up study* Am. J Digest. Dis. 20 152-156, June 1953.

After massive resection of the small intestine, a patient suffered from persistent diarrhea, and three and a half years after operation macro
Intramuscular administration of

a day brought about a maximum reticulocyte response of 19 7 per cent on the eighth day Treatment was continued with injection of 15 mcg. twice a week, and the diarrhea responded dramatically Blood pressure, which had become low after surgery became elevated again after correction of the anemia with vitamin B_{12}.

Indianapolis General Hospital
Indianapolis, Ind.

421 BETHELL, F H. *Anemias resulting from disturbances of gastrointestinal function*, Rev Gastroenterol. 19 890-896, Nov 1952.

Excerpts from this general article follow "Anemias resulting from disturbances of gastrointestinal function include the hypochromic microcytic anemias due to iron deficiency and the macrocytic normochromic anemias caused by lack of folic acid or Vitamin B_{12} and perhaps by other as yet unidentified substances. The daily iron requirement of an adult male is about 1 mg., that of a menstruating female and a rapidly growing child prior to adolescence is approximately 2 mg. The pathogenesis of the macrocytic anemias with megaloblastic marrow reaction has been greatly elucidated within recent years. It is now known that both folic acid and Vitamin B_{12} are required for normal hemopoiesis and that they participate in wide-spread metabolic processes.

Vitamin B_{12} is now known to be but one of a group of related compounds for which the generic term 'cobalamin has been proposed. The Vitamin B_{12} analogues are present chiefly in sources of animal protein but it is probable that in man appreciable quantities may become available through intestinal synthesis. On the other hand, certain bacteria, including coliform organisms, require for their growth an exogenous source of B_{12} and so may deprive the host of the vitamin. It has been conclusively demonstrated that the dietary extrinsic factor of Castle is Vitamin B_{12}. The chemical nature of the intrinsic factor has not been elucidated. Evidence has recently been presented which indicates that intrinsic factor may combine with Vitamin B_{12} to form a microbiologically inactive complex, thereby in some manner facilitating the absorption of the vitamin. Deficiency of Vitamin B_{12} may result from dietary lack of cobalamin although this appears to be a less common cause of nutritional macrocytic anemia than deficiency of folic acid. Chronic intestinal disorders, including idiopathic steatorrhea, disseminated ileitis, partial obstruction and diminished absorptive surface resulting from short circuiting or resection of small intestine may lead to impaired absorption to both Vitamin B_{12} and folic acid. Patients who survive for several years after total or subtotal gastrectomy are prone to develop a syndrome indistinguishable from pernicious anemia. Vitamin B_{12} usually constitutes a complete form of replacement therapy for pernicious anemia, when administered intramuscularly in doses of 10 to 30 micrograms at intervals adjusted to individual needs. Treatment of macrocytic megaloblastic anemias other than pernicious anemia is directed primarily toward correction, if possible, of the underlying disorder Alleviation of folic acid deficiency usually may be accomplished by the oral administration of 10 mg. daily The parenteral route should be reserved for patients with intestinal disease. Orally administered Vitamin B_{12} in daily dosage of 5-10 mcg. may be effective in correcting macrocytic anemia due to dietary lack of this vitamin when pernicious anemia can be excluded with reasonable certainty "

In the discussion Dr Bethell is asked why folic acid sometimes aggravates neurologic symptoms in pernicious anemia he replies "Regarding the effect of folic acid in aggravating the neurologic damage in pernicious anemia, I feel that larger doses of folic acid in the absence of B_{12} seem to cause true progression of the lesion on the other hand, we have been impressed by the fact that in the anemias of pregnancy the administration of B_{12} appears to aggravate the clinical and hematologic manifestations of folic acid deficiency Animal experiments show that there is a mutual relationship between B_{12} and folic acid, and in deficiencies of both vitamins, when they are created, the administration of one will be effective for a while but later there will be a rapid aggravation of the deficiency of the one that is not supplied we do know that Vitamin B_{12} is apparently essential for the integrity of the nervous system "

University of Michigan
Ann Arbor, Mich.

422. HALL, B. E.: *Diagnosis and treatment of nutritional anemia*, J.A.M.A. 151 1-8, Jan. 3, 1953.

A review with 45 references on the diagnosis and treatment of nutritional anemias, such as iron deficiency anemias, pernicious anemia, nontropical sprue, megaloblastic anemia of infancy and of pregnancy and the puerperium, and anemias associated with disease and surgery of the gastrointestinal tract. Nutritional anemias are rather rare in the North Central section of the United States and when seen are usually due to faulty absorption and assimilation of food, rather than to inadequate intake of food. Experiences with and indications for the newer hematopoietic agents, such as vitamin B_{12} and folic acid, are discussed.

There is no justification for the administration of vitamin B_{12} or folic acid to patients with iron deficiency anemia these patients need iron, yet are often treated with vitamin B_{12} and folic acid, a needless expense.

Parenteral vitamin B_{12} or liver extracts continue to be the preferred means of treating pernicious anemia. Oral administration of large amounts of vitamin B_{12} without concomitant administration of gastric juice (which contains intrinsic factor) can elicit a significant hematopoietic response, but the response is usually suboptimal, as illustrated by a patient who was given 1,000 mcg. of vitamin B_{12} every second day A total of 32,000 mcg. of vitamin B_{12} was given in three months before the erythrocyte count rose to more than 4 million per cu. mm. A similar response could be obtained in eight weeks with 5 mcg. of vitamin B_{12} by mouth daily plus 150 cc. of human gastric juice. This case illustrates the importance of intrinsic factor in potentiating the absorption and utilization from the alimentary tract of the small amounts of vitamin B_{12} normally present in foodstuffs. The author emphasizes the fact that folic acid alone should not be used to treat pernicious anemia. Hematologic response is incomplete and the neurologic manifestations of pernicious anemia may develop or progress when the patient receives folic acid only

In nontropical sprue, absorption of many nutrients is impaired. Therefore, vitamin B_{12} and folic acid do not

relieve manifestations of the disease that are unrelated to deficiency in hematopoietic substances. Recently other investigations have shown cortisone to be of value in nontropical sprue, apparently by improving the efficiency of intestinal absorption. Cortisone did not improve the hematologic findings therefore, hematopoietic agents should be given along with cortisone.

Megaloblastic anemia of infancy is due mainly to folic acid deficiency Its combined treatment with folic acid and ascorbic acid is recommended. The ascorbic acid is apparently necessary for conversion of folic acid into folinic acid. It is mentioned that the incidence of megaloblastic anemia of infancy has diminished since ascorbic acid has been added to proprietary milk products. Megaloblastic anemia of pregnancy and the puerperium does not respond to vitamin B_{12} but does respond to folic acid.

It is pointed out that megaloblastic anemia rarely follows total gastrectomy Apparently the stomach is not the only source of intrinsic factor Megaloblastic anemia sometimes occurs in patients operated on for regional enteritis; 3 such patients are described, one of whom responded to intramuscular vitamin B_{12} (10 mcg. daily) and the others to folic acid.

Stanford University School of Medicine
San Francisco, Calif.

ANIMAL STUDIES

423 BORCH-MADSEN P., and SØEBORG OHLSEN A.: *On the effect of total resection of the fundus ventriculi in thriving swine upon the antianemic factor content of liver* Acta med. Scandinav (supp. 213) 131 91 98, 1948.

Copenhagen, Denmark

424 CAMERON D G., WATSON G. M., and WITTS, L. J: *The experimental production of macrocytic anemia by operations on the intestinal tract*, Blood 4 803-815, July 1949

425. DYKE, W J C., and HIND H. G *Bacterial synthesis of vitamin B_{12} in the alimentary tract*, Lancet 1 486-488, March 18, 1950.

The authors found that the vitamin B_{12} growth-promoting activity for L. lactis Dorner and L. leichmannii in meat is not increased by incubation with normal gastric juice.

A table is given for the amounts of vitamin B_{12} present in the alimentary canal at various levels in man, horse, and sheep it is present in greatest amount below the ileocecal valve.

Purified extracts made from intestinal contents are clinically active in the treatment of pernicious anemia when given parenterally

The authors suggest that in man the normal requirements of vitamin B_{12} are met by bacterial synthesis in the colon. They state that some advantage is undoubtedly to be obtained from supplementation with foodstuffs rich in vitamin B_{12}.

Evans Biological Institute
Runcorn, Cheshire England

426. HAWK, E. A., and HUNDLEY J M. *Effect of certain B vitamin deficiencies on gastric secretion in the rat*, Proc. Soc. Exper Biol. & Med. 78 318-322, Oct. 1951

The effect of vitamin B deficiencies on gastric secretion was investigated in rats subjected to ligation of the pylorus. Acid secretion was sharply reduced by pyridoxine deficiency but there was marked increase in the volume of secretion when pyridoxine was given about 16 hours before the beginning of the secretory test. These findings indicate that pyridoxine plays a previously unsuspected part in the secretory process. Thiamine deficiency produced a marked depression in acid secretion, while riboflavin and pantothenic acid deficiencies produced only slight depressions. In the acute reversal experiments, supplementation with the missing vitamin caused an insignificant rise in the animals deficient in thiamine but not in those deficient in riboflavin. Neither aureomycin nor vitamin B_{12} had any effect on gastric secretion when added to the complete basal diet, although each caused a slight increase in weight gain. Pepsin secretion was not greatly decreased in any of the deficiencies studied, with the possible exception of thiamine. Pyridoxine deficiency increased both concentration and total output of pepsin.

National Institutes of Health
Bethesda, Md.

427 BERK, L., CASTLE, W B., WELCH, A. D., HEINLE, R. W., ANKER, R., and EPSTEIN M.: *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. X Activity of vitamin B_{12} as food (extrinsic) factor* New England J Med. 239 911 918 Dec. 9 1948.

Four patients with untreated pernicious anemia were given 5 mcg. of vitamin B_{12} dissolved in 125 or 150 cc. of physiologic saline solution, daily by mouth, for 10 days. Immediately thereafter they were given the same amount of vitamin B_{12} daily with 125 or 150 cc. of previously neutralized normal human gastric juice for a second 10-day period. One patient was given 5 mcg. of vitamin B_{12} a day intravenously during a third period. The reticulocyte rise was low or absent when vitamin B_{12} was given alone but was present in each case when gastric juice also was given, and in 3 of the patients the reticulocyte response was accompanied by a significant increase of red cells.

These observations indicate that the hemopoietic activity of orally administered vitamin B_{12} in pernicious anemia is potentiated by simultaneous administration of normal human gastric juice, but that parenterally administered vitamin B_{12} is still more effective. It is suggested that the food (extrinsic) factor may be identical with or closely related to the anti-pernicious anemia principle of liver which presumably is identical with vitamin B_{12}, and that the gastric (intrinsic) factor is necessary for the optimal utilization of the relatively small amounts of vitamin B_{12} or related substances present in various foods. Preliminary findings in an untreated patient with pernicious anemia suggest that the fecal elimination of vitamin B_{12} is so great as to create a deficiency which cannot be abolished by the amount of the vitamin derived from the food or synthesized by intestinal bacteria. Other observations suggest that 70 per cent alcoholic extracts of beef muscle, a classic source of extrinsic factor contain a substance possessing both microbiologic activity as vitamin B_{12} and hemopoietic activity when intravenously injected in patients with pernicious anemia. It is therefore possible that the function of the intrinsic factor of normal gastric juice is to facilitate absorption by the intestine of vitamin B_{12} or of chemically related compounds in the food, rather than to react with the extrinsic factor as has hitherto been assumed.

Boston City Hospital, and
Harvard Medical School
Boston, Mass.
Western Reserve University and
University Hospitals
Cleveland, Ohio

428. LANDBOE-CHRISTENSEN E., and PLUM, C. M.: *Localization of the anti-anemic principle in the human stomach. Anti-anemic effect of powderized human fundus,* Acta med. Scandinav (suppl. 206) 130 309-310, 1948 (in Soc. Proc.)

429 BOCCUZZI, G. and PAOLINO W: *Regulation of hematopoesis. Importance of the antipernicious (anemia) factor in the mechanism of digestive leucocytosis,* Minerva med. 391 139-143, 1948 (abstr C. A. 42 6910b, Sept. 20, 1948)

Clinical data show a positive correlation between HCl secretion and increase in leukocytes, and between ingested HCl and similar leukocytes, in addition to the phenomenon of retarded leukocytosis, and the capacity of different foods to produce different degree of leukocytosis, according to their content of extrinsic factor

430. GARDNER, F H., HARRIS J W., and CASTLE, W B.: *Erythropoietic activity of extrinsic factor on parenteral administration in pernicious anemia,* Am. J Med. 7 421, Sept. 1949 (in Soc. Proc.)

Castle at first believed that interaction between the food (extrinsic) and the gastric (intrinsic) factors was necessary for the formation of the anti-pernicious anemia principles of liver—here considered to be vitamin B_{12}. However this reaction does not occur in vitro. The erythropoietic effect of orally administered vitamin B_{12} has been shown to be enhanced by the simultaneous administration of the intrinsic factor although the same amount of vitamin B_{12} given alone parenterally is still more effective.

In the present observations a beef muscle extract was inert when administered daily by mouth to 3 patients with untreated pernicious anemia. When administered orally every day with 150 cc. of normal human gastric juice it was moderately active, and when administered intravenously without gastric juice it was still more active. Microbiologic assays and erythropoietic effects indicate the presence of somewhat less than 1 microgram of vitamin B_{12} in each daily dose of the beef muscle extract.

These observations suggest that the extrinsic factor is identical with or closely related to vitamin B_{12} and that the intrinsic factor is essential merely to facilitate absorption of low concentrations of vitamin B_{12} present in certain foods other than, for example, liver

431 MORGAN E. H., HALL, B. E., and CAMPBELL, D C.: *Hematopoietic activity of parenterally administered beef muscle concentrate in cases of pernicious anemia,* Proc. Staff Meet., Mayo Clin. 24 594-597 Nov 23, 1949

Since other investigators have shown that beef muscle functions as extrinsic factor the question arose whether its activity might be due to the presence of vitamin B_{12}. Three patients with pernicious anemia in relapse were treated with 20 mg. of beef muscle concentrate a day intramuscularly the amount being derived from 125 Gm. of beef muscle and having approximately 1 mcg. of vitamin B_{12} activity as determined by microbiologic assay The hematopoietic responses of 2 of these patients appeared to be optimal, and the response of the third was suboptimal. Since the hematopoietic activity of beef muscle concentrate parenterally administered appears to be similar to that of liver extracts, it is presumably due to the presence of vitamin B_{12}. It seems highly probable that the extrinsic factor in beef muscle is identical with vitamin B_{12}.

Mayo Foundation
Rochester Minn

432. CAMPBELL, D C., HALL, B. E., and MORGAN E. H.: *Oral administration of vitamin B_{12} in pernicious anemia. II Studies on the nature and source of intrinsic factor* Proc. Central Soc. Clin. Research 22: 18, Nov 1949 J Lab. & Clin. Med. 34 1590 Nov 1949 (in Soc. Proc.)

Since vitamin B_{12} orally administered depends for its effect on the presence of intrinsic factor (which is believed to be absent in patients with pernicious anemia) the authors made an intensive study of the intrinsic factor in 17 patients with pernicious anemia in relapse. By keeping the quantity of vitamin B_{12} constant, they were able to test various unknowns for the presence or absence of intrinsic factor The following observations were recorded:

(1) Intrinsic factor is present in fresh, Berkefeld filtered, pooled human gastric juice and (2) also in extracts of hog stomach and duodenum. (3) It is destroyed by heating at 63 C. for half an hour (4) It is not lost in human gastric juice with a pH of 1 7 when stored for three months at a temperature of 5 C. (5) Trichloracetic acid and cold alcohol (95%) precipitates from human gastric juice did not contain the intrinsic factor (6) In vitro mixtures of pooled human gastric juice and vitamin B_{12} that had stood at room temperatures and then been heated to 70 C. for half an hour produced no hemopoietic response when given orally to a patient with pernicious anemia. (7) Although the minimal amount of human gastric juice required to produce optimal hemopoietic responses when administered daily by mouth with 5 or 10 mcg. of vitamin B_{12} was not determined in these experiments, it was found that 75 cc. produced the best results. (8) When large quantities of vitamin B_{12} alone were administered by mouth, the response varied widely one patient showed no hemopoietic effect after a single dose of 1,000 mcg. of vitamin B_{12} concentrate another showed a suboptimal effect after a single dose of 75 mcg. of crystalline vitamin B_{12}.

These experiments and similar findings by other investigators suggest that the lack of intrinsic factor in patients with pernicious anemia may not be absolute.

Rochester Minn.

433. GARDNER, F H., HARRIS, J W., SCHILLING R. F., and CASTLE, W B. *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XI Hematopoietic activity in pernicious anemia of a beef muscle extract containing food (extrinsic) factor upon intravenous injection without contact with gastric (intrinsic) factor* J Lab. & Clin. Med. 34 1502 1511, Nov 1949

Seven patients with addisonian pernicious anemia were given 10 cc. of a 70 per cent alcohol extract of 400 Gm. of beef muscle (as a source of extrinsic factor) by mouth, daily for 10 days or longer Only one of them showed a detectable reticulocyte response. When four of the patients received the extract together with 150 cc. of normal human gastric juice, all of them showed reticulocyte response. Subsequently the extract was given to 3 of the patients intravenously without additional gastric juice a further reticulocyte response was observed. The hematopoietic effect of the daily intravenous injection of 10 cc. of beef muscle extract was shown to be less than that of the daily intramuscular injection of 1 mcg. of crystalline vitamin B_{12}. Ten cc. of the beef muscle extract was shown by microbial assays to contain 0.37 to 0.9 mcg. of vitamin B_{12} activity The authors state that the hematopoietic activity of the beef muscle extract when given orally with gastric juice is surprisingly great, judging from observations of the potentiation by normal human gastric juice of 5 mcg. of crystalline vitamin B_{12}. It is therefore suggested that there are substances in beef extract other than vitamin B_{12} which are potentiated by normal human gastric juice upon oral administration in pernicious anemia.

The gastric (intrinsic) factor was not observed to increase the hematopoietic action of pteroylglutamic acid, nor did it enhance the intestinal absorption of glucose, l-tyrosine, or the digestion products of casein.

Thorndike Memorial Laboratory
Boston City Hospital, and
Harvard Medical School
Boston, Mass.

434 HALL, B. E., BETHELL, F H., MORGAN, E. H., CAMPBELL, D C., SWENDSEID M. E., MILLER, S., and CINTRON RIVERA, A. A. *Observation on the presence of intrinsic factor in extracts of hog stomach and duodenum,* Proc. Staff Meet., Mayo Clin. 25 105-113, March 1 1950

Twelve patients with pernicious anemia in relapse were treated with vitamin B_{12} given by mouth and variously prepared fractions of stomach and duodenum of swine as a source of intrinsic factor The daily dosage of vitamin B_{12} was 5 or 10 mcg. and that of the intrinsic factor varied from 0.92 to 8.0 Gm. Extracts of hog duodenal mucosa appear to have greater potentiating activity on vitamin B_{12} administered orally than do extracts of the entire duodenum.

A thirteenth patient was treated with an acid extract of ground duodenum and vitamin B_{12} in the form of a complex supplied in capsules each of which contains 9 mcg. of vitamin B_{12} and the principle present in 0.33 Gm. of acid extract of ground duodenum. The hematopoietic response was fairly satisfactory although probably suboptimal, and clinical improvement occurred but was not as dramatic as that in patients receiving vitamin B_{12} parenterally This complex possesses greater heat stability than does the intrinsic factor alone.

Mayo Clinic and Mayo Foundation
Rochester Minn.
Simpson Memorial Institute
Ann Arbor Mich.
Wayne County General Hospital
Eloise, Mich.

435. LEADING ARTICLE *Pernicious anaemia and the intrinsic factor* Brit. M. J 2 612-613 Sept. 9 1950

Pernicious anemia is discussed with relation to Castle s intrinsic factor The presence of this factor is necessary for absorption of orally administered vitamin B_{12} from the gastrointestinal tract. The nature of the reaction between the intrinsic factor and vitamin B_{12}, and its specificity and function are as yet not understood. It is believed that the intrinsic factor s action is enzymic. Evidence is cited which shows that several substances are capable of binding vitamin B_{12}, making it [illegible] gastrointestinal microorganisms. However only

teraction with intrinsic factor is absorption of vitamin B_{12} from the intestinal tract increased. It is believed that in pernicious anemia the greatly reduced production of intrinsic factor is a *result of gastric atrophy* and that the vitamin B_{12} in food is consequently not modified or absorbed.

436. UNGLEY C. C.: *Absorption of vitamin B_{12} in pernicious anaemia. II Oral administration with normal gastric juice*, Brit. M. J 2 908-911, Oct. 21 1950.

Author's summary and conclusions "The effect of vitamin B_{12} given with normal gastric juice was observed in eight patients with pernicious anaemia. The total amounts were usually 50 µg. + 500 ml. given as a single dose or in divided doses.

"One patient failed to respond to oral therapy In one who received 40 µg. with only 150 ml. of gastric juice the response was very poor In two the oral-dose/parenteral-dose ratio was about 10:1 suggesting that about 10% of the vitamin had been absorbed. In the remaining four the response was about as good as would have been expected if the same dose of vitamin B_{12} had been injected. Thus small doses of vitamin B_{12} given with normal gastric juice are sometimes efficiently absorbed.

"In these eight experiments each 100 ml. of gastric juice seemed to be enough for the absorption of 0, less than 1, 1 less than 2, 5 or 10, 10 10 and 10 µg. of vitamin B_{12}. Thus the amount of gastric juice necessary to promote the absorption of a given amount of B_{12} varied considerably These variations are perhaps only partly due to differences in the amount of intrinsic factor in the samples of gastric juice differences in the recipients may have been equally important."

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

437 UNGLEY C. C., and CHILDS, G. A. *Absorption of vitamin B_{12} in pernicious anaemia. III Failure of fresh milk or concentrated whey to function as Castle's intrinsic factor or to potentiate the action of orally administered vitamin B_{12}*, Brit. M. J 2 911-915, Oct. 21, 1950.

Five cases of pernicious anemia were treated with orally administered vitamin B_{12}, together with fresh milk (2 patients) or whey concentrates (3 patients) The concentration of vitamin B_{12} used was 10 mcg. in 500 cc. of milk. The whey concentrate had a total volume of approximately 650 cc. which contained 50 mcg. of vitamin B_{12}. Effects of these combinations were compared with those of one or more of the following parenterally given vitamin B_{12}, normal gastric juice plus B_{12}, B_{12} plus folic acid, and folic acid plus milk. The results on the blood picture were such that the authors made the following conclusions "Neither *fresh milk nor a* whey concentrate in the doses given was an adequate substitute for normal human gastric juice as a source of Castle's intrinsic factor"

Royal Victoria Infirmary
Newcastle-upon-Tyne, England
Glaxo Laboratories
Greenford, Middlesex, England

438. UNGLEY C. C. *Absorption of vitamin B_{12} in pernicious anaemia. IV Administration into buccal cavity into washed segment of intestine, or after partial sterilization of bowel*, Brit. M. J 2 915-919 Oct. 21, 1950.

Studies were undertaken to determine whether vitamin B_{12} would be effectively absorbed if the possible destructive action of intestinal juice could be reduced or avoided.

When 5 mcg. of vitamin B_{12} was applied daily to the buccal mucosa of a patient with pernicious anemia, there was a negative response. This patient subsequently responded to 5 mcg. of B_{12} given orally with gastric juice.

In four experiments in 2 patients, the instillation of vitamin B_{12} with or without gastric juice into a washed segment of intestine isolated between two balloons of a Miller-Abbott tube resulted in negative or trivial responses. Each patient subsequently responded to vitamin B_{12} given parenterally or orally with gastric juice.

In a fourth patient, vitamin B_{12} was given orally after preliminary partial sterilization of the intestine with aureomycin, dihydrostreptomycin, and phthalylsulfathiazole. Even 80 mcg. of B_{12} was ineffective. This patient later responded to the same dose of B_{12} given orally with gastric juice. In his conclusion, the author states that these negative findings are against, but do not entirely exclude, the possibility that Castle's intrinsic factor acts by protecting vitamin B_{12} from destruction in the gastrointestinal tract.

Royal Victoria Infirmary
Newcastle-upon-Tyne, England

439 SCHILLING R. F., FRUTON J. S., HOFSTEE, B. H J., WELCH, A. D., HARRIS, J W., GARDNER, F H., and CASTLE, W B.: *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XII Failure of thymus aminopolypeptidase to act as intrinsic factor* J Lab. & Clin. Med. 36 942-949 Dec. 1950.

In 3 patients with pernicious anemia, daily oral doses of 5 mcg. of vitamin B_{12} were not potentiated by active preparations of aminopolypeptidase derived from calf thymus, but were potentiated by neutralized normal human gastric juice in amounts of either 10 or 150 cc. daily This evidence indicates that aminopolypeptidase from calf thymus does not possess intrinsic factor activity as does neutralized normal human gastric juice. The small amount of normal human gastric juice needed to potentiate vitamin B_{12} suggests that in persons without pernicious anemia the usual clinical methods for determining the volume of gastric secretion would not be adequate to establish or to deny the physiologic adequacy of intrinsic factor

440. SUÁREZ, R. M., PÉREZ, E., and NAZARIO R. *The failure of urogastrone as a source of the intrinsic factor* Bol. Asoc. méd. de Puerto Rico 43 618, 1951 (abstr Blood 7 759 July 1952)

"In the hope that some of the gastric juice intrinsic factor may leave the body in the urine of normal persons, the authors tried the simultaneous oral administration of vitamin B_{12} in 30 µg. daily doses and urogastrone in

doses of 0.15 Gm. daily on a typical case of pernicious anemia showing signs and symptoms of combined system disease. The dialyzed polysaccharide urogastrone failed as a potentiating factor for orally administered vitamin B_{12}.

"Subsequently the case responded well both hematologically and neurologically to the oral use of folic acid in 5 mg. doses three times a day plus the parenteral administration of vitamin B_{12} in 30 µg. daily doses. The case proves that folic acid can be administered safely to pernicious anemia patients showing nervous system involvement if vitamin B_{12} is given parenterally at the same time and that urogastrone does not contain Castle's intrinsic factor."

441 SCHILLING R. F., HARRIS, J. W., and CASTLE, W. B. *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XIII Hematopoietic activity of vitamin B_{12a} (vitamin B_{12b})* Blood 6 228-232, March 1951

The authors have studied the hematopoietic response to vitamin B_{12a}, administered both intramuscularly and orally in 9 patients with addisonian pernicious anemia. In 2 patients comparisons were made with intramuscular vitamin B_{12}, and in 2 more cases, comparisons were made with oral vitamin B_{12}. The intramuscular dosage of vitamin B_{12a} ranged from 1 to 4 mcg. a day the oral dosage was 5 mcg. a day given first with 50 cc. of water and then with 50 cc. of normal human gastric juice. It was found that vitamin B_{12a} is as potent a hematopoietic agent as vitamin B_{12} when administered parenterally to patients with pernicious anemia in relapse. It was further found that the hematopoietic activity of vitamin B_{12a}, like that of vitamin B_{12}, and of vitamin B_{12a}, is potentiated by simultaneous oral administration with normal human gastric juice. One of the patients had mild subacute combined degeneration of the spinal cord. Vitamin B_{12a} (1 mcg. daily for 16 days) produced improvement.

Thorndike Memorial Laboratory
Boston City Hospital, and
Harvard Medical School
Boston, Mass.

442. BETHELL, F. H., SWENDSEID M. E., MILLER, S., and CINTRON RIVERA, A. A.: *Cobalamin (vitamin B_{12}) and the intrinsic factor of Castle* Ann. Int. Med. 35 518-528, Sept. 1951

A number of chemically distinct forms of vitamin B_{12} have been isolated. The generic name proposed for the entire group is cobalamin. This appears to be the dietary or extrinsic factor of Castle. Individual members of the cobalamin group have been designated as cyanocobalamin (vitamin B_{12}) and hydroxycobalamin (vitamin B_{12b}). All the cobalamin products tested clinically thus far appear to possess the same type and degree of hemopoietic activity

The efficacy of liver extracts in the treatment of pernicious anemia appears to depend upon their content of cobalamin, and solutions of cobalamin given parenterally are effective substitutes for liver extracts. Orally administered cobalamin, however is not efficiently utilized by patients with pernicious anemia, as is shown by a case report. An exogenous source of intrinsic factor is required in such cases when desiccated hog duodenal mucosa was given the patient responded. Another patient resp to a heated trypsin digest of an extract of duo mucosa which had been incubated with 5 mcg. of c cobalamin.

In severe macrocytic anemia there may be a d deficiency of cobalamin which can be completel rected by the daily oral administration of 5 mc crystalline cyanocobalamin. Reports of 2 patient macrocytic anemia are presented one of these pa did well on oral cyanocobalamin without other m tion; the other who represented a complicated pict dietary deficiency responded better to parenteral th oral administration, and responded better still wher acid was given intramuscularly in addition to coba

The part played by intrinsic factor in potent orally administered cobalamin is not understood. I not appear to protect the vitamin from destruction alimentary tract nor to prevent its utilization by ba in the stomach and small intestine. There seems to parallelism between the intrinsic factor activity of g and duodenal preparations and their capacity to co with cobalamin, thus preventing the vitamin from s lating the growth of microorganisms which require

University of Michigan
Ann Arbor Mich.
Wayne County General Hospital
Eloise, Mich.

443 GLASS G B. J., BOYD L. J., RUBINS M. A., and SVIGALS, C. S. *Relationship of dular mucoprotein from human gastric ju Castle's intrinsic antianemic factor* Science 101 108, Feb. 1, 1952.

Nine patients who received vitamin B_{12} ora intramuscularly and who also received mucoprotein observed in an effort to understand better the natu Castle's intrinsic factor (Apparently vitamin B_{12} *extrinsic factor.)* Measurements were made of the r locytes, red blood cells, hematocrit, and hemoglol the blood and megaloblasts in bone marrow The au review the pertinent literature (36 references) and to the following conclusion " it cannot be sta present whether glandular mucoprotein is the pure i sic factor or whether the intrinsic factor (enzyme contained in or adsorbed to its molecule. Howev the present stage of information, taking into ac normal occurrence of glandular mucoprotein in th man gastric juice, the rather homogeneous appearan this substance on electrophoresis, the extent to wh fits into physiological and pathological data availal intrinsic factor the close similarity of physical ch teristics of mucoprotein and intrinsic factor an potent intrinsic factor activity of mucoprotein, it ap permissible to consider glandular mucoprotein fr of the gastric dissolved mucin as the main carrier o intrinsic factor activity of the human gastric juice

New York Medical College,
Flower and Fifth Avenue Hospitals, and
Metropolitan Hospital Research Unit
New York, N. Y.

444 GLASS G B. J., BOYD L. J., and SVIG C. S.: *Potentiation of hematopoietic effect of administered vitamin B_{12} by glandular mucopr of the human stomach,* Federation Proc. 11 March 1952.

"The hematopoietic response of patients with macrocytic anemia to small doses of vitamin B_{12} administered orally is known to be poor or inconsistent, although the same patients usually respond well to parenteral administration. It has been postulated that this discrepancy was due to the absence of Castle's intrinsic factor from the stomach of patients with pernicious anemia and the consequently deficient absorption of B_{12} from the gastrointestinal tract. In earlier studies the authors showed that one of the components of gastric dissolved mucin, glandular mucoprotein from fundal glands of human stomach closely resembled intrinsic factor in physical and physiological properties. Moreover this substance was found consistently absent or present only in traces in gastric juice of patients with pernicious anemia. In 7 patients with this disease oral doses of 10-30 mcg. B_{12} daily were uniformly ineffective. When 50-200 mg. of glandular mucoprotein daily was added to B_{12}, however a full hematopoietic response occurred. Recently 2 patients with nutritional macrocytic anemia whose gastric juice contained a normal concentration of mucoprotein were studied. In these patients daily administration of 10-30 mcg. of B_{12} alone by mouth was followed by a prompt hematopoietic response to normal. These findings suggest the possibility that the presence of glandular mucoprotein in gastric juice may be required for small doses of orally administered vitamin B_{12} to be effective. Moreover the quantitation of glandular mucoprotein in gastric juice may be of value in the differentiation of various types of macrocytic anemia capable of responding to B_{12}."

New York Medical College, and
Flower and Fifth Avenue Hospitals
New York, N Y

445. TOWNSEND S. R. *Pernicious anaemia due to deficiency of extrinsic factor* Canad. M. A J 67 53-54, July 1952.

A case of pernicious anemia believed to be the result of deficiency in protein which caused deprivation of the extrinsic factor was successfully treated with vitamin B_{12} by mouth.

Montreal General Hospital
Montreal, Canada

446. MERSHEIMER, W L., GLASS, G B., SPEER, F D., WINFIELD J M., and BOYD L. J: *Gastric mucin—a chemical and histologic study following bilateral vagectomy gastric resection and the combined procedure,* Ann. Surg. 136 668-679 Oct. 1952.

The effect of surgical procedures (partial gastrectomy bilateral vagectomy or both) on the pattern of hydrochloric acid and glandular mucoprotein secretion following insulin or histamine stimulation was studied.

In the discussion on this article, Dr Glass points out that disturbances in the secretion of glandular mucoprotein following vagectomy and gastrectomy may be of significance in interpreting the hematologic disturbances which may or may not occur after operations on the stomach. Dr Glass' studies indicate that glandular mucoprotein is closely related to Castle's intrinsic hematopoietic principle. It is present in all normal gastric juices but is absent or present only in traces in the stomach of patients with pernicious anemia. Normal individuals respond to administration of insulin with a rise in mucoprotein and acid concentration, but in patients with pernicious anemia the response of the acid as well as that of glandular mucoprotein to vagal stimulation is abolished. In studies on 9 patients with pernicious anemia in relapse it has been shown that glandular mucoprotein probably is needed for the utilization of orally administered vitamin B_{12}. In the absence of mucoprotein the oral ingestion of vitamin B_{12} is practically without hematopoietic effect. However when glandular mucoprotein processed from normal human gastric juice is added to small oral doses of B_{12}, a hematopoietic effect is obtained. Glandular mucoprotein is produced by the fundic glands of the stomach. Thus in patients with partial gastrectomies in which the fundus is preserved, an ensuing macrocytic anemia need not be expected since mucoprotein would still be secreted. After total gastrectomy on the other hand, no mucoprotein is available. This is probably why macrocytic anemias have been noted with increasing frequency after total gastrectomy in patients not treated with liver or vitamin B_{12} injections, provided that they do not eat liver and live for more than three years. After this period the stores of the hematopoietic principle become exhausted and macrocytic anemia may develop.

New York Medical College
New York, N Y

447 MARMION, B. P., GARDNER, H. J., SAINT E. G., and STÜBBE, J L. *Gastric mucoprotein and intrinsic factor response of pernicious anaemia to oral vitamin B_{12} plus normal gastric juice treated with receptor-destroying enzyme,* Lancet 1 273-274, Feb. 7 1953.

Authors summary "A young woman with classical pernicious anaemia in relapse was treated with oral vitamin B_{12} [10 mcg. per day] and human gastric juice acted upon by receptor-destroying enzyme (R.D.E.) A normal therapeutic response was obtained.

"Although there is good evidence that intrinsic factor in gastric juice is a mucoprotein, it is not one which is inactivated by R.D.E., thereby differing in its properties from gonadotrophin and mucoproteins derived from respiratory-tract cells."

Walter and Eliza Hall Institute of Medical Research, and
Royal Melbourne Hospital
Melbourne, Australia

448. GLASS, G B. J *Hematopoietic activity of glandular mucoprotein from human gastric juice,* Gastroenterology 23 219-233, Feb. 1953.

Author's conclusions "Glandular mucoprotein, secretory product of mucous cells of the stomach, acts as a potentiator of the hematopoietic activity of vitamin B_{12} on its oral intake. It is yet undetermined by which mechanism it facilitates the intestinal absorption of vitamin B_{12} and whether glandular mucoprotein is identical with Castle's intrinsic hematopoietic factor or whether it acts as its carrier to which Castle's factor is bound.

"The participation of glandular mucoprotein in the metabolism of vitamin B_{12} does not preclude the existence of other factors influencing the effects of vitamin B_{12} upon hematopoiesis, such as folic-folinic acid axis or the role of the specific inhibitor of erythrocytes maturation in pernicious anemia.

"With the absence of glandular mucoprotein from the stomach, which is the case in pernicious anemia, the hematopoietic effect of vitamin B_{12} administered by mouth in small or moderate doses is very weak or none. The addition of glandular mucoprotein to by itself inefficient small oral doses of B_{12} results in a striking hematopoietic response. With the presence of glandular mucoprotein in the gastric juice in patients with macrocytic nonpernicious anemia the oral administration of small doses of B_{12} alone may cause a hematopoietic response, provided that the cases are suitable for the treatment with B_{12}.

"The conclusive evidence for the significance of the presence of glandular mucoprotein in the stomach for the ability of patients with macrocytic anemia to respond to the oral treatment with B_{12} is yet to be obtained. It appears, however that the presence or absence of glandular mucoprotein in the gastric juice may be helpful in differentiation of various forms of macrocytic anemias."

New York Medical College and Flower and Fifth Avenue Hospitals New York, N. Y.

449 WALLERSTEIN, R. O., HARRIS J W., SCHILLING, R. F., and CASTLE, W B. *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XV Hematopoietic effects of simultaneous intravenous and of simultaneous or serial oral administration of intrinsic factor and vitamin B_{12}.* J Lab. & Clin. Med. 41 363-375, March 1953.

Authors summary and conclusions: "Observations were made upon fifteen patients with pernicious anemia under controlled dietary conditions. No consistent evidence was obtained for potentiation of the hematopoietic effect of vitamin B_{12} by simultaneous daily intravenous injection with intrinsic factor A reciprocal rather than a stoichiometric relation exists between vitamin B_{12} and intrinsic factor with respect to the potentiation of vitamin B_{12} by intrinsic factor upon simultaneous daily oral administration.

"The hematopoietic effect of the daily oral administration of 1 μg. of vitamin B_{12} may be detectably increased by as little as 10 ml. of normal human gastric juice. However the hematopoietic effect of 1 μg. of vitamin B_{12} and 50 ml. of gastric juice does not equal that of the daily injection of 1 μg. of vitamin B_{12} alone. When separated by an interval of three or four hours the hematopoietic effect of the serial administration of vitamin B_{12} and of intrinsic factor is greater when the intrinsic factor precedes the vitamin B_{12}. These and other recent clinical observations suggest that the essential physiologic function of intrinsic factor is merely to increase somewhat the assimilation of vitamin B_{12}. They indicate the possibility that the primary effect of intrinsic factor is upon the intestinal wall rather than upon the vitamin B_{12}."

Boston City Hospital, and Harvard Medical School Boston, Mass.

450. PRATT P T., and JOHNSON M. O.: *Pathogenesis and treatment of pernicious anemia,* Nebraska M. J 38 84, 87 March 1953.

A review of the literature on the pathogenesis of pernicious anemia shows the defect to be an inability to absorb vitamin B_{12} from the gastrointestinal tract. Maturation of the nucleus of the red blood cell involves the changing of amines into thymine, which is potentiated by the folic acid–folinic acid mechanism, and the change from thymine to thymidine, which is potentiated by vitamin B_{12}. The formation of hemoglobin is dependent on iron metabolism. Vitamin B_{12} deficiency slows nuclear growth—larger but fewer red cells are formed there are also fewer leukocytes and thrombocytes, and nerve cell metabolism may be affected. The life span of the red cell is apparently reduced when vitamin B_{12} is inadequate.

The authors have seen 11 patients with pernicious anemia treated with vitamin B_{12} at the University Hospital and Hematology Clinic since January 1 1950. In patients in relapse, clinical improvement has been maintained by the equivalent of 1 or 2 mcg. of vitamin B_{12} a day administered as a total dose every two to three weeks. In neurologic pernicious anemia the dose is increased in amount and frequency until no further improvement is obtained or until evidence of neurologic disease is gone, usually for more than a year after which the patient is placed on a maintenance dose.

University of Nebraska College of Medicine Omaha, Neb.

ANALYTICAL STUDIES

451. TERNBERG J L., and EAKIN R. E.: *Erythein and apoerythein and their relation to the antipernicious anemia principle* J Am. Chem. Soc. 71 3858, Nov 1949

Normal gastric juice contains a nondialyzable, heat labile substance (apoerythein) which combines with erythrotin (vitamin B_{12}) to form a nondialyzable complex (erythein) In this combination erythrotin is not available to microorganisms, but it is released by heat. Hog gastric mucosa preparations contain a principle which appears to be analogous to apoerythein. The principle has been isolated and studied. It is probable that apoerythein is the intrinsic factor of Castle s or a component thereof.

University of Texas and Clayton Foundation for Research Austin, Texas

452. WOLF D E., WOOD T R., VALIANT J., and FOLKERS K. *Vitamin B_{12} and the intrinsic factor* Proc. Soc. Exper Biol. & Med. 73 15-17 Jan. 1950.

Studies of possible chemical reaction between crystalline vitamin B_{12} and gastric juice showed no evidence for such a reaction. No evidence of gross structural change in vitamin B_{12} by reaction between this vitamin and the gastric juice intrinsic factor was found. A possible reaction resulting in a very small structural change of the large vitamin B_{12} molecule, which could produce *as little as 5 mcg. of orally active* product, is being investigated. A concentrate prepared from beef muscle having vitamin B_{12}-like microbiologic activity was found to have hemopoietic activity when administered parenterally to pernicious anemia patients thus, the

factor acts like vitamin B_{12}. These results support the previous hypothesis that the effect of the intrinsic factor is due to its ability to promote the absorption of the small amounts of vitamin B_{12} or its equivalent which occur in natural foods.

Merck & Co., Inc.
Rahway, New Jersey

453. MEYER, C. E., EPPSTEIN S. H., BETHELL, F H., and HALL, B. E. *Nature of the intrinsic factor* Federation Proc. 9 205, March 1950.

"During the course of concentrating the intrinsic factor from hog intestines, certain similarities between this factor and lysozyme became apparent. It has been observed that lysozyme derived from egg white has the property of combining with vitamin B_{12}, thus rendering it unavailable to Lactobacillus lactis Dorner and Escherichia coli. Oral administration of 9 µg. of vitamin B_{12} in the form of the complex, daily to patients with pernicious anemia results in remission, with characteristic reticulocyte response and erythropoiesis."

The Upjohn Company
Kalamazoo, Mich.
Simpson Memorial Institute
Ann Arbor Mich.
Mayo Clinic
Rochester Minn.

454 RIDING D: *Vitamin B_{12}*, Brit. M. J 2 348, Aug. 5, 1950 (in Soc. Proc.)

It is suggested that in man the normal requirements of vitamin B_{12} are met by bacterial synthesis in the colon, and that many of the fecal organisms may be capable of synthesizing this vitamin.

The author states that normal gastric juice contains a nondialysable, heat-labile substance which combines with vitamin B_{12} to form a complex which is nondialysable and not dissociated by dialysis. The B_{12} in this complex is unavailable to microorganisms, but, upon being released by heat, is again microbiologically active. This inhibitor is believed to be Castle's intrinsic factor or a fraction thereof. The intrinsic factor has been found in large quantities in all specimens of human gastric juice containing free hydrochloric acid. Pernicious anemia patients do not have it. Of the animals, the dog and the pig possess the intrinsic factor the horse, calf and sheep do not.

Since orally given vitamin B_{12} produces a response in cases of pernicious anemia, it is possible for man to use this vitamin without the presence of this intrinsic factor It is conjectured that the intrinsic factor may act as a protective mechanism. It is suggested that the complex formed by B_{12} and the intrinsic factor might also prevent destruction of this vitamin by the alkaline digestive juices of the upper intestine.

Rasmore, England

455. HALL, B. E., MORGAN, E. H., and CAMPBELL, D C.: *Studies on the nature of the intrinsic factor of Castle*, Brit. M. J 2 585-589 Sept. 9 1950.

In the therapy of pernicious anemia, orally administered vitamin B_{12} requires for its effective utilization the presence of a potentiating agent (intrinsic factor of Castle) In the absence of this factor hemopoietic responses are variable and unpredictable when the vitamin is given orally in doses up to 100 times the minimal effective parenteral dose. Known sources of the intrinsic factor include gastric juice of normal or elevated acidity from human beings, and extracts of the stomach and intestines of swine. It has not been possible to prepare concentrated preparations which yield consistently effective and reliable therapeutic results because of ready destructibility during processing The authors believe that their studies support the concept of the enzymic nature of the intrinsic factor

Three hypotheses are presented to explain the role of the intrinsic factor which is probably concerned with the absorption of vitamin B_{12} 1) it may alter the chemical or physical nature of vitamin B_{12} so that it traverses the intestinal barrier more readily 2) it may protect the vitamin from destruction by secretions of the digestive tract, and 3) it may prevent the removal of the vitamin from the contents of the upper part of the gastrointestinal tract by microorganisms. Certain observations are cited with respect to the third hypothesis 1) In cases of pernicious anemia a great increase has been found in gastrointestinal tract concentrations of Escherichia coli and Clostridium welchii. 2) Normally certain levels of the small intestine are relatively free of bacteria. 3) Recently Davis and Mingioli (personal communication) have shown that E. coli has an avidity for vitamin B_{12}. 4) Lichtman, Ginsberg, and Watson (unpublished data) have reported that the daily oral administration of 3 Gm. of aureomycin and 3 mcg. of vitamin B_{12} to patients with pernicious anemia in relapse resulted in a hemopoietic response. Since aureomycin would be expected to reduce the number of colonic organisms in the gastrointestinal tract, it is believed that vitamin B_{12} may be made more available for absorption.

Mayo Clinic
Rochester Minn.

456. NOER, B. *Bacterial synthesis of vitamin B_{12}*, Lancet 2: 706, Dec. 2, 1950 (in Letters to the Editor)

The writer reports bacteriologic investigations of the use of mucosa of fresh pig stomach and of three commercial preparations of pig stomach against pernicious anemia. A constant finding has been the presence of only two types of bacteria in considerable numbers—Bacillus subtilis and one of the nonpathogenic corynebacteria. The writer presumes that one of these bacteria may be identical with the "intrinsic factor" Both bacteria have been found to synthesize vitamin B_{12} in significant amounts. It is stated that, although clinical investigations with strains of these bacteria in cases of pernicious anemia have not given convincing results, these bacteria are able to synthesize not only vitamin B_{12}, but also antibiotics which are instrumental in the control of undesirable bacterial growth in the intestinal mucosa in these cases.

The following working hypothesis of the etiology of pernicious anemia is proposed: "If vitamin B_{12} is to be absorbed it must be present in the intestinal mucosa in a concentration higher than in the tissues. This is the case in the normal organism, where the intestinal mucosa is infected with B_{12}-producing strains. In the diseased organism these strains are expelled from the mucosa and replaced by B_{12}-consuming *Bact. coli* and lactobacilli,

and this mucosa forms a barrier to the absorption of B_{12} from the intestinal contents."

Further investigations are under way

Ferrosan, Ltd.
Copenhagen, Denmark

457 BEERSTECHER, E., JR. *A biologically active product of vitamin B_{12}*, Federation Proc. 10 161, March 1951.

"Thermal treatment of crude sources of intrinsic factor produces an intrinsic factor-uncombinable thermolabile factor which stimulates the growth of bacteria in the presence of vitamin B_{12} under assay conditions which have hitherto been found to produce a response only to vitamin B_{12}. This response is considerably greater than that produced by similar amounts of the vitamin. The data suggest that this factor is a normal product of vitamin B_{12} and is probably the erythrocyte maturation factor (EMF) Since the factor is inactive in the absence of the vitamin, it appears that vitamin B_{12} has more than a single metabolic product critical for growth. The evidence indicates that intrinsic factor combines with vitamin B_{12} to produce a complex, the erythrotin of Eakin and Ternberg (*J Am. Chem. Soc.* 71 3858, 1949 [Abstr 451]) which is normally enzymatically degraded to form the active principle, thus explaining the inability of vitamin B_{12} synthesized in the gut to manifest a hematopoietic response. The chemical properties of the active principle are being characterized."

University of Texas
Houston, Texas

458. BIRD O D., and HOEVET B. *The vitamin B_{12}-binding power of proteins*, J Biol. Chem. 190 181 189 May 1951.

Authors summary "A variety of proteins, including intrinsic factor concentrate and lysozyme, bind vitamin B_{12} when measured by adding them unheated in increasing amounts to tubes of vitamin B_{12} assay medium containing known amounts of vitamin B_{12} and noting the inhibition of growth of Lactobacillus leichmannii.

"This inhibition method indicated that intrinsic factor concentrates bind vitamin B_{12} compounds but not thymidine, whereas lysozyme binds vitamin B_{12} and thymidine.

"A method of measuring vitamin B_{12}-binding based on the non-dializability of the bound vitamin indicates a much higher capacity of intrinsic factor concentrate to bind vitamin B_{12} than is shown by the inhibition method. This dialysis method indicated that lysozyme does not truly bind vitamin B_{12}, whereas intrinsic factor concentrate does, since the added vitamin is dialyzed from the *former but not the latter* The method is also more suitable than the inhibition method for determining the vitamin B_{12}-binding power of crude sources of active protein which contain relatively large amounts of non-vitamin B_{12} growth factors for L. leichmannii."

Parke, Davis and Co.
Detroit, Mich.

459 GORDIN, R.: *Studies on Castle's extrinsic factor* Acta. med. Scandinav 140 199- July 23, 1951 (abstr J.A.M.A. 147 1890, Dec. 1, 1951)

"To trace and produce in the purest possible form a substance acting as an *extrinsic factor experiments* were performed on 17 patients with cryptogenetic pernicious anemia who were given substances other than ordinary meat by mouth, with gastric juice used as the intrinsic factor The substances used as the extrinsic factor were peptone, fibrin hydrolysate (aminosol®) crude meat extract, pure meat extract, raw ground liver liver extract ("heptomin") and vitamin B_{12}. A good effect was obtained with peptone, different meat extracts, small quantities of liver and liver extract, and small doses (5 mcg.) of vitamin B_{12}. Tests with fibrin hydrolysate gave less satisfactory results. The amount of intrinsic factor contained in 40 ml. of normal gastric juice proved sufficient as a daily dose. Chemical analyses of fibrin hydrolysate and peptone demonstrated that these substances do not contain cobalt. Consequently the part played by cobalt in the extrinsic factor effect, as suggested by Smith and Rickes, with respect to vitamin B_{12}, seems to be debatable."

460. EAKIN R. *Apoerythin*, Chem. & Engin. News 29 5450 Dec. 24, 1951 (in Soc. Proc.)

At the seventh ACS Southwest Regional Meeting "Apoerythin was the name applied to a protein factor which binds vitamin B_{12}. The important characteristic of this material is that it may be administered orally in pernicious anemia cases where previously injection of antianemia principles has been required."

461. CALLENDER, S. T., and LAJTHA, L. G. *On the nature of Castle's hemopoietic factor* Blood 6 1234-1239 Dec. 1951

Experiments in vitro support the theory that Castle's hemopoietic factor is a thermolabile combination of normal gastric juice (intrinsic factor) and vitamin B_{12} (extrinsic factor) In vitro a combination of crystalline B_{12} and gastric juice as well as normal serum matured megaloblasts *to normoblasts. Neither* gastric juice nor crystalline B_{12} alone affected megaloblasts. It was also found that the hemopoietic in normal serum is thermolabile.

Radcliffe Infirmary
Oxford, England

462. HOFF JØRGENSEN E. *The effect of "intrinsic factor" on the absorption of vitamin B_{12} by wild type of Escherichia coli*, Arch. Biochem. & Biophysics 36 235-236, 1952 (abstr Blood 7 857 Aug. 1952)

"Although wild type E. coli does not require vitamin B_{12} as a growth factor the organism will absorb a large amount of B_{12} from the medium. Extracts of pig stomach inhibit this absorption. Lysozyme is without effect. This may offer an explanation of the effect of 'intrinsic factor as it is known that in patients suffering from pernicious anemia large amounts of E. coli are found in the upper intestine and even in the stomach."

University of Copenhagen
Copenhagen, Denmark

463. WELCH, A. D., SCHARF V., HEINLE, R. W., and MEACHAM, G. C.: *Assay for intrinsic factor in patients with pernicious anemia in remission given radioactive vitamin B_{12}* Federation P 308-309 March 1952.

"Orally administered vitamin B_{12} produces little or no hematopoietic effect in patients with pernicious anemia unless intrinsic factor is administered simultaneously The presence of materials with vitamin B_{12}-activity presumably formed by bacteria in the intestine, has precluded the determination in the feces of unlabeled ingested vitamin. Studies with cyanocobalamin-Co^{60} (Merck & Co.) the gamma radiation of which can be measured in the presence of feces by scintillation counting, have shown that patients with pernicious anemia *in remission* given oral doses of only 0.5 µg. (0.03 µc.) of the radioactive vitamin, excrete 70-95% of the cobalt in the feces. When gastric juice or concentrates of hog stomach are administered simultaneously with the vitamin, the amount of cobalt excreted is reduced to 5-30% of that ingested. A typical patient given 3 doses of radioactive vitamin B_{12} (0.5 µg.) orally without intrinsic factor eliminated 70, 95 and 95% respectively of the cobalt concurrent administration of 10 mg. of a hog stomach fraction reduced the elimination to 25% and a greater effect was not produced by a dose of 100 mg The findings in all patients suggest that intrinsic factor is involved in the removal of the vitamin from the gastrointestinal contents and form the basis of a technique for assay of intrinsic factor fractions. The methods are simple and relatively rapid as compared with the classical assay procedure requiring prolonged clinical and hematologic observations in seriously ill, untreated patients."

Western Reserve University School of Medicine
Cleveland, Ohio

464 BURKHOLDER, P R.: *Microbiological studies on materials which potentiate oral vitamin B₁₂ therapy in addisonian anemia*, Arch. Biochem. & Biophysics 39 322-332, Aug 1952 abstr J Clin. Nutrition 1 171, Jan. 1953.

Author's summary "Strains of bacteria were isolated from stomach and jejunal juice of addisonian anemia patients, and most of these bacteria were found to have a strong tendency to absorb vitamin B_{12} from solution. Concentrates prepared from hog stomach mucosa when added to incubating mixtures prevented the consumption of vitamin B_{12} by the bacteria. Our studies support the theory that typical addisonian anemia is a disease caused by removal of vitamin B_{12} from the patient's digestive tract by a greatly increased microbial flora under conditions of achlorhydria and lack of intrinsic factor in the gastric region.

"Preparations of swine mucosa showing high potency in rendering vitamin B_{12} unavailable to bacteria are also rich in intrinsic factor activity as determined with adequate doses administered along with vitamin B_{12} orally to patients, with resultant characteristic hematopoietic responses. Any conclusion concerning the identity of vitamin B_{12} absorption factor with the intrinsic factor of Castle awaits chemical purification and demonstration of both microbiological and clinical potency in the purified material."

The mode of action of both vitamin B_{12} and intrinsic factor is described and substantiated by 19 references.

Osborn Botanical Laboratory
Yale University
New Haven, Conn.

465 CHOW B. F., and YAMAMOTO R.: *Properties of B_{12} binding substance in gastric juice*, Federation Proc. 12 189 March 1953.

"A substance *(X)* in gastric juice forms with vitamin B_{12}, a complex not easily dissociable by dialysis or absorption with resting microorganisms. This complex could be tagged isotopically with B_{12} labeled with Co^{60} thus facilitating separation by discarding fractions without radioactivity Such studies yielded the following results *a) Physio-chemical characteristics.* The complex was soluble in 40% aqueous alcohol but completely precipitable at 60% It possessed a minimum solubility at pH 3.5-4.0. Isoelectric precipitation yielded a purified fraction with an approximate molecular weight of 5×10^{6} by diffusion measurement. It contained more than one fraction by paper chromatography *b) Importance of acidic group in B_{12} binding* The presence of strong acidic groups in *(X)* suggests the possibility of other compounds with similar groups to bind with B_{12}. Sulfonated polysaccharides, hydrocarbons or proteins were also capable, to varying degrees, of reducing the uptake of B_{12} by microorganisms and releasing it from tissues and, in some instances, aided in the absorption. *c) Binding powers of gastric juice from individuals with pernicious anemia and from old subjects.* Radioactive B_{12} bound to a given amount (N or volume) of gastric juice varied quantitatively according to the source of the latter Gastric juice from subjects with pernicious anemia (adult or infantile) lacked B_{12} binding power and that from certain groups of the aged (65 years or older) combined with only a small amount, when compared with healthy adults."

Johns Hopkins University
Baltimore, Md.

466 GLASS G B. J: *Gastric mucin and its constituents physicochemical characteristics cellular origin, and physiological significance*, Gastroenterology 23 636-658, April 1953

This article is devoted mainly to the chemistry and physiology of gastric mucin. Glandular mucoprotein is a constituent thereof. Brief mention is made of 7 patients with pernicious anemia who showed no or very slight hematologic response to daily administration of 10 to 30 mcg. of oral vitamin B_{12} daily When 50 to 200 mg. of glandular mucoprotein was added daily to the same oral dose of vitamin B_{12}, a strong hematopoietic response was obtained.

New York Medical College
New York, N Y

467 SWENDSEID M E., SHAPIRO H., and HALSTED J A. *Missing link may be pernicious anemia factor* Science News Letter 63 296, May 9 1953.

"A missing link in a nutritional chain involved in the manufacture of red blood cells may be an important factor in pernicious anemia.

"In the research a substance known as the B-12 binding factor which is present in the gastric and duodenal contents of normal subjects, could not be found in the duodenum of persons with pernicious anemia.

"Although the exact role of the binding factor is still unknown, it is thought that it may be essential in the

absorption and utilization of vitamin B-12. Vitamin B-12 plays an important role in the manufacture of red blood cells, and thus the lack of the binding factor could interfere with this process.

"The study also indicated that persons who have had their entire stomachs removed by surgery may not be able to absorb enough B-12 for normal nutrition. The binding factor was not found in duodenal contents of such patients examined in the investigation. "

University of California at Los Angeles Medical School, and
Los Angeles VA Center
Los Angeles, Calif.

468. PRUSOFF W H., WELCH, A. D., HEINLE, R. W., and MEACHAM, G. C.: *Concentration of intrinsic factor and vitamin B_{12}-binding activities of fractions of desiccated hog stomach*, Blood 8 491 501 June 1953.

Authors summary "Fractionation of desic fatted hog stomach through the use of isoelec ammonium sulfate precipitation technics, and g assays in patients with pernicious anemia in rela daily oral doses of 5 μg of vitamin B_{12}, has le concentration of intrinsic factor activity in an a protein equivalent to 0.03 per cent of the origin of crude material. The electrophoretic pattern active fraction indicated a lack of homogeneity

"The vitamin B_{12}-binding activities of three obtained by *precipitation with ammonium sulf* investigated by microbial inhibition and dialysis There was no correlation between the ability of tions to bind vitamin B_{12} and to exert intrinsi activity *Among these fractions, that with the* intrinsic factor activity in patients with pernicio in relapse bound the least amount of vitamin

Western Reserve University School of Medicine, and
University Hospitals
Cleveland, Ohio

Nutrition, Growth and Metabolism

VITAMIN DEFICIENCY

469 BETHELL, F H. *Nutritional factors concerned in blood formation*, Chicago M. Soc. Bull. 51 710-711, March 12, 1949

One of the nutritional factors briefly reviewed in this article is vitamin B_{12}. Its relationship to folic acid is not yet clear. The deficiency of these vitamins in the body is followed by macrocytic anemia and granulocytopenia. It may be that the conjugated form of folic acid supplied by food must be converted to the free form by specific enzymes or conjugates before it can be utilized, and that in patients with pernicious anemia there is a relative inability to convert or split the conjugated to the free form. The feces of patients with pernicious anemia contain relatively large amounts of vitamin B_{12}, which may mean that the vitamin is synthesized by bacteria in the bowel where absorption does not occur or that there is a specific absorption defect in the patients. The author concludes "It seems possible that the extrinsic factor of Castle is a dietary complex containing vitamin B_{12} in a conjugated form or as a precursor substance and that the intrinsic factor is an enzyme concerned with the conversion of the vitamin into a form in which it can be absorbed or utilized."

470. SPIES, T D: *The control of pellagra*, Rhode Island M. J 32: 431-435, Aug. 1949

This paper includes a discussion of nutritional macrocytic anemia. Vitamin B_{12} is said to be the most effective anti-anemic substance known, per unit of weight. It is effective in pernicious anemia, nutritional macrocytic anemia, and tropical sprue, diseases which are often associated with pellagra.

Hillman Hospital
Birmingham, Ala.

471. WISSMER, B.: *Les facteurs alimentaires dans l'hématopoïèse (Alimentary factors in hematopoiesis)* Presse méd. 57 898-900, Oct. 15, 1949

The author reviews the various dietary elements which take part in the elaboration of hemoglobin and erythrocytes. Iron, copper and folic acid are usually present in sufficient amounts, but in Biermer's anemia (pernicious anemia) the organism cannot transform folic acid from its conjugated form to a utilizable form. Vitamin B_{12} may be the extrinsic factor. Its action appears to be very powerful. The other vitamins are necessary in general but not individually; this is also true for amino acids. The role of proteins has been demonstrated, particularly in severe burns, anemia of pregnancy and tropical anemia. In conclusion, the author suggests that the proteins supplied in the diet seemingly are reduced to simple substances, stored and resynthesized in special organs such as the liver. It is probably from this source of endogenous proteins that amino acid precursors of the globin molecules and protein stromas are formed.

Lausanne, Switzerland

472. MORRIS, J E., HARPUR, E. R., and GOLDBLOOM, A.: *The metabolism of l-tyrosine in infantile scurvy* J Clin. Investigation 29 325-335, March 1950.

Increased urinary excretion of tyrosine and its deaminated derivatives, tyrosyl and hydroxyphenyl, was demonstrated in 6 male infants with scurvy or preclinical scurvy following the ingestion of tyrosine (1 Gm./Kg. body weight/day incorporated in a formula providing approximately 3.5 Gm. of cow's milk protein/Kg. daily) The urinary excretion of hydroxyphenyl varied. Darkening was observed in the urine of 2 subjects when the hydroxyphenyl excretion was minimal. Ascorbic acid given intramuscularly (500 mg./day) decreased the urinary excretion of tyrosyl compounds although tyrosine feeding was continued.

Pteroylglutamic acid, 45 mg. intramuscularly given concurrently with tyrosine ingestion inhibited tyrosyluria.

Vitamin B_{12}, 30 mcg. intramuscularly failed to influence the urinary excretion of tyrosine derivative whether given as a single injection during an established hydroxyphenyluria or twice a day concurrently with tyrosine feeding. Serum tyrosyl values were elevated during periods of tyrosyluria. Serum amino nitrogen values paralleled the changes in serum tyrosyl content.

Children's Memorial Hospital, and
McGill University
Montreal, Canada

473. WADA, S., and ARAKAWA, T *Blood picture of nutritional dystrophy in children effect of folic acid and vitamin B_{12} upon it (Studies on the nutrition of children in Hirosaki area, 10th report)* Tohoku J Exper Med. 56 47 June 25, 1952 (abstr J Am. Dietet. A. 29 64, Jan. 1953)

"Blood examinations were made of a group of Japanese children, who, though apparently healthy showed signs of the pellagrous form of nutritional dystrophy The effects of folic acid and of vitamin B_{12} upon the blood picture in these cases were also observed. Macrocytic anemia was found in 35 cases of the 46 examined in winter and in only 5 cases of the 38 examined in the summer When 15 mg. folic acid were injected subcutaneously each day for two or three successive days, the majority of cases showed an increase in red blood cells, a reticulocyte response, a return to normal volume index, a decrease in white cells, and decreased eosinophile count, and a slight reduction in color index. The response to vitamin B_{12} was minimal. Decrease in white cell count or in eosinophile count was observed in only a minority of cases treated with the vitamin."

GROWTH STUDIES

CHILDREN

474 WETZEL, N C, FARGO W C, SMITH, I. H., and HELIKSON J : *Growth failure in school children as associated with vitamin B_{12} deficiency—response to oral therapy* Science 110 651-653, Dec. 16, 1949

The growth-promoting properties of vitamin B_{12} have been demonstrated in certain bacteria and in animals. Since the dietary depletion method used in animals cannot be employed with children, it is necessary to observe the effects of vitamin B_{12} under normal growth-promoting conditions in states of simple growth failure. These conditions include good hygiene, balanced exercise and rest, planned caloric conservation, emotional calm, and a satisfactory diet. One of the authors (Wetzel) had previously developed statistical methods by which the growth trends of individual children can be charted, and curves of expected growth plotted and compared with the actual subsequent growth. Wetzel's Grid is constituted of measures of various aspects of growth.

Vitamin supplements and whole liver extract given orally have frequently had beneficial effects in cases of simple growth failure in which delay in progress has been out of proportion to physical or other findings. However progress sometimes is unaccountably slow and this has suggested the existence of unknown deficiencies. In August 1949 the authors began administering 10 mcg. of crystalline vitamin B_{12} each day orally to 11 children aged 5 to 12 years, 3 of whom were making slow progress and 8 of whom were selected at random from a larger group under regular care for varying degrees of malnutrition and in varying states of recovery from simple growth failure. All routine or special treatment a child had been receiving, such as extra rest or oral whole liver extract, was continued.

Five of the 11 children responded dramatically to this single change in their routine, as shown statistically on the Wetzel Grid. Their growth responses over a period of eight weeks were equivalent to those to be expected from a further 100 to 240 days of regular institutional care without the help of B_{12}. In the authors words, the results "speak with measurable statistical certainty of what may be termed B_{12} functional deficiency that was definitely benefited by oral therapy"

The most dramatic general effects of vitamin B_{12} were shown by a boy with severe allergic bronchitis, whose sleep had been interrupted regularly for 12 months by asthmatic attacks and whose desire and ability to eat had been greatly diminished by wheezing. The growth response in this case was accompanied by a remarkable attenuation of the asthmatic symptoms, which completely vanished during the first week of B_{12} administration.

After B_{12} administration the only noticeable clinical changes were increased physical vigor and alertness, better general behavior and greatly improved appetite. A moderate eosinophilia (6 to 8%) and reticulocytosis (0.6 to 0.9%) were found in 9 of the 11 children.

Children's Fresh Air Camp and Hospital
Cleveland, Ohio

475. CHOW B. F *Sequelae to the administration of vitamin B_{12} to humans*, J Nutrition 43 323-343, Feb. 1951.

The effect of vitamin B_{12} on the weight gain of chronically ill and clinically healthy children was studied. The chronically ill children were of various ages and had diseases such as rheumatic heart disease, malnutrition, anemia, and mental retardation. The normal children were 18 males ranging in age from 18 to 47 months. Both ill and normal children were given 25 mcg. of crystalline vitamin B_{12} daily by mouth. Babies under 2 years of age received 10 mcg. of vitamin B_{12} daily Older children received tablets and younger ones, a solution of the vitamin in milk. Control children in the chronically ill group received no placebo (since they were already getting many pills) while healthy children serving as controls received a placebo solution in their milk. All children were weighed regularly

In the chronically ill group, after three months of vitamin B_{12} administration the mean gain in body weight in the experimental group was practically twice that in the control group. The difference in mean weight gains began to manifest itself after one month, and increased consistently with time. Among the normal children, the mean gain in body weight of the children in the B_{12} group was consistently greater than that of the controls from the fourth week on. At the end of 24 weeks, the supplemented group showed a weight gain of 58 ounces, and the controls, a gain of 40 ounces.

However because of the heterogeneity of the ill children and because of the small number of normal children studied, the author considers the results as merely suggestive, although it is stated that the weight differences are statistically significant. The author states, however that "with an adequate diet, there is no reason to expect very dramatic effects from B_{12} supplementation."

Oral administration of vitamin B_{12} over a period of several months or in a single massive dose (5.0 mg. per adult) did not result in the appearance of vitamin B_{12} activity in the urine. A major portion of the ingested vitamin B_{12} appeared in the feces. These results indicate poor but apparently adequate absorption of vitamin B_{12} from the gastrointestinal tract. When vitamin B_{12} was given intravenously to man, the blood level decreased rapidly and the administered vitamin could be recovered in the urine. The vitamin was not absorbed by or did not penetrate into, the erythrocytes in vivo or in vitro. When vitamin B_{12} was given parenterally to infants fed on a soybean protein diet, no increase in nitrogen retention occurred.

Johns Hopkins University
Baltimore, Md.

476. BENJAMIN, B., and PIRRIE, G. D *Effect of vitamin B_{12} on underweight children*, Lancet 1 264, Feb. 2, 1952 (in Letters to the Editor)

An extensive and carefully controlled study by the London County Council failed to confirm the favorable results obtained by Wetzel (*Science* 110 651, 1949 [Abstr. 474]) from the use of oral vitamin B_{12} in r underweight after long illness or from malnutrition.

dosage used was 10 mcg. daily of a product supplied by Glaxo. Vitamin B_{12} affected neither growth nor the incidence of minor ailments.

London County Council
London, England

477 WETZEL, N. C., HOPWOOD H. H., KUECHLE, M. E., and GRUENINGER, R. M. *Growth failure in school children. Further studies of vitamin B_{12} dietary supplements*, J Clin. Nutrition 1 17 Sept.-Oct. 1952 (abstr J Am. Dietet. A. 29 164 Feb. 1953)

"Group studies were designed to determine how effective vitamin B_{12} supplementation for overcoming simple growth failure would be in the natural home environ ment. On the nutritional side, simple growth failure involves both substance as well as fuel debts which must be paid before recovery is achieved. Results of B_{12} supplementation are shown in tables. Twenty children in one group received B_{12} supplements for 16 weeks in another group, 16 children were given the same supplement for 6 weeks. Of the total of 36 children, 23 responded defi nitely to the treatment by significant growth gain. No differences were found between crystalline and concen trated forms of the vitamin. From the standpoint of a nutrition program, the study reveals general benefits well beyond the point of individual recovery responses."

478. WETZEL, N C., HOPWOOD H. H., KUECHLE, M. E., and GRUENINGER, R. M. *Growth failure in school children further studies of vitamin B_{12} dietary supplements*, J Clin. Nutrition 1 17-31, Sept.-Oct. 1952.

Vitamin B_{12} has been shown to exert a "growth promoting effect" when given to children in growth failure. From the regular public school enrollment lists of Shaker Heights, Ohio, children whose growth records showed a state of growth failure were selected for treatment and study Oral vitamin B_{12} supplements were given daily at the schools. (Double doses were given on Monday and Friday and none on Saturday and Sunday) Dosage was 10 mcg. daily for 6 or 16 weeks. Sixteen of the 20 chil dren in the 16-week program and 7 of the 16 in the 6-week program had an increased rate of growth after receiving the vitamin B_{12} supplements. The net rate of gain for each group changed from below to above normal, showing that the children who responded were gaining part of the growth which should have been but was not gained in previous months. Other benefits accrued to the students, such as less fatigue, greater power of concen tration, and better all-around progress than before.

In a further study involving 236 children and giv ing similar results, crystalline vitamin B_{12} and concen trate vitamin B_{12}, both given orally were shown to be equivalent in growth-promoting effect.

Nutrition records of 45 children in the program showed that those who responded to vitamin B_{12} supplements had a distinct advantage over the nonresponders in caloric intake, food choice and diet composition.

The growth pattern of one student is described in detail and her response to vitamin B_{12} shown in the grid pattern.

Children's Fresh Air Camp and Hospital,
Shaker Heights Public Schools, and
Western Reserve University
Cleveland, Ohio

479 WILDE, E. *The treatment of growth failure in Aleut school children*, J Pediat. 40 565-569 May 1952.

A study was made, using the Wetzel Grid technic, of the effect of a proprietary nutritional supplement hav ing B_{12} activity (2.0 mcg./cc.) on the growth and development of 9 retarded Aleut children, aged 10 to 14 years.

Wetzel Grid records were available for several years on these children and they were selected because of a slowdown in the rate of development. During a treatment period of seven months the nutritional supplement was given at school in doses of 5 cc. daily on the first four days and 10 cc. on the fifth. Since the children had previously received multivitamin tablets, it was felt that "improvement in the rate of growth and development in the treated children would probably be due to the added growth-stimulating effect of vitamin B_{12}."

The results are described as follows "During the period of treatment there was a striking increase in the rate of growth in seven of the nine subjects, as measured in Grid levels per month the growth rate in terms of developmental levels per month was restored, from a control rate of 0.71 before treatment to 1.02 levels per month. This is a value that coincides with the standard rate of 1 level per month characteristic of all healthy children of school age. In considering only the seven children who responded, the growth rate during the test period was 1.14 levels per month. This indicates that the growth failure had been stopped and that the previous lag was being corrected. The increase in the rate of growth and development is 65.2 per cent. These findings are consistent with the reports of Wetzel and associates, indicating that in some children growth failure occurs as a manifestation of functional deficiency of vitamin B_{12} and that it may be corrected by oral administration of vitamin B_{12}."

St. Paul Island
Alaska

480. WILDE, E.: *The growth of Aleut infants*, Canad. M. A. J 68 70-71, Jan. 1953.

Daily administration of 1 cc. of a proprietary nutritional supplement increased the rate of growth, as measured by the Wetzel Grid, of 7 Aleut infants who had been gaining normally before the experiment. The supplement contains, per cc., 0.8 mg. riboflavin, 5.0 mg. ferrous sulfate, and has the activity of 2.0 mcg. of vitamin B_{12}. Since prior to the experiment the infants had been given all these vitamins except vitamin B_{12}, the increase in growth rate must be attributed to the single new factor The author regards it of interest that the growth-stimulating effect of such a small amount of vitamin B_{12} (2.0 mcg. daily) can be detected by the Wetzel Grid.

Chinle, Ariz.

481. RESSA, E., and SORAGNI, E. *La vitamina B_{12} quale fattore di accrescimento (Vitamin B_{12} as growth factor)* Acta paediat. lat. 4 311-320, May June 1951

482. ROSSI, A. *La vit. B_{12} in terapia quale fattore di sviluppo ed agente anabolizzante (Vitamin B_{12} in therapy as a growth factor and anabolic agent)* Policlin. Sez. prat. 58: 1099-1100, Aug. 27 1951

483. REVIEWS *Growth promotion by vitamin B_{12} in children*, Nutrition Rev 8 139-141 May 1950.

484 BIDAULT *Activité de la vitamine B_{12} par voie orale chez l'enfant en que facteur de croissance (Activity of vitamin B_{12} given orally as a growth factor in the child)* Concours méd. 73 3673-3674 Nov 3, 1951.

485. THERAPEUTICS *Hepatoterapia en el crecimiento infantil (Liver therapy in growth of children)* Orientacion Med. 1 602, April 17 1953.

Thirty-two children were given 1.20 Gm. of liver powder in tablet form daily for 18 weeks 28 other children served as controls. There was a weight gain of 285 Gm. more and a 0.6 cm. greater increase in growth in the group that received liver than in the control group. It is stated that these results would indicate that the dietary factor which influences growth is liver and not vitamin B_{12}, as commonly believed.

INFANTS

486. SALMI, L. *Effect of vitamin B_{12} in chronic nutritional disturbances in infants*, Clin. Pediat. 32 617 Nov 1950 (abstr A.M.A. Am. J Dis. Child. 83 93, Jan. 1952)

"Five hypotrophic infants showed dramatic improvement clinically after the administration of vitamin B_{12} (7 γ daily) Under this treatment the blood levels of protein, amino acids, iron, and cholesterol reached normal levels."

487 CASTLE, W B., ELVEHJEM, C. A., MAY C. D., WELCH, A. D., ZUELZER, W W., and BUTLER, A. M. *Vitamin B_{12} and folic acid in infant nutrition*, J.A.M.A. 146 1028-1029 July 14, 1951.

The adequacy of vitamin B_{12} and of folic acid in milk diets customarily fed to infants was discussed in a report to the A.M.A. Council on Foods and Nutrition. Evidence was presented indicating that human milk contains between 0.1 and 1.0 mcg of vitamin B_{12} and folic acid per liter Cow's milk contains slightly more folic acid and definitely more vitamin B_{12} than human milk. It was agreed that infants fed artificially with cow's milk formulas would receive as much vitamin B_{12} and folic acid as breast fed infants, because these formulas usually contain 50 to 70 per cent cow's milk. Thus, there appears to be no need to supplement with vitamin B_{12} and folic acid the diet of normal infants being fed cow's milk formulas.

The conference group did not believe that there was evidence of widespread deficiency of vitamin B_{12} and folic acid among infants and children as revealed by megaloblastic anemia or suboptimal growth. A cautious attitude is assumed toward using vitamin B_{12} or folic acid in an attempt to augment normal growth of healthy infants being fed breast milk or cow's milk formulas. Certain pathologic conditions causing reduced intake or impaired assimilation in infants might increase the need of vitamin B_{12} and folic acid. However the group did not feel that evidence presented justified widespread prophylactic use of vitamin B_{12} or folic acid in order to meet the extra requirements of the occasional pathologic situation. A deficiency of ascorbic acid does cause an increased need of folic acid. Folic acid apparently serves as a precursor of the citrovorum factor and ascorbic acid enhances the production of this factor from folic acid. The evidence available does not indicate that vitamin B_{12} is critically involved in the conversion of folic acid to citrovorum factor but it may be concerned with the subsequent formation, from this factor of another metabolically important compound.

488. MENEGHELLO J: *Progress of hospitalized malnourished infants*, J.A.M.A. 150 1505-1506, Dec. 13, 1952 (in Foreign Letters, Chile)

In a letter describing the treatment, mainly dietary of 1,539 malnourished hospitalized infants, it is mentioned that "To modify anemic conditions of the malnourished infants vitamin B_{12} was given, producing a very satisfactory response in 75% of the cases."

489 BIDAULT G *Activité de la vitamine B.12 par voie buccale, chez l'enfant en tant que facteur de croissance (Activity of vitamin B_{12} given orally as a growth factor in the child)* Arch. Hospitalieres: 401-403 (No. 12) 1952.

Regardless of the cause and other treatment received, all undersized children in the author's practice were given 50 mcg. of vitamin B_{12} orally per day in two doses. This regimen was continued without interruption for 6 to 12 months, until "normal" values for height and weight were obtained. It was noted that in general weight gains began almost immediately while changes in height did not occur until after three months of therapy

In the follow-up it was shown that when the children had been undersized because of endocrine disease or infection, their growth rate had decreased so that three months after the last dose of vitamin B_{12} they were on the verge of becoming undersized for their age but not under weight for their size. When the original cause of small size was endocrine malfunction, the children had retained the gains made and were growing normally three or six months later

It is suggested that vitamin B_{12} can establish the endocrine equilibrium necessary for ideal growth, and is a useful adjuvant to hormonal therapy (thyroid, somatotropin, testosterone, follicular hormone, androstenediol)

PREMATURE AND NEWBORN INFANTS

490. DOWNING, D F *Failure of vitamin B_{12} to promote growth of premature infants*, Science 112 181 Aug. 11, 1950 (in Comments and Communications)

Of 48 premature infants with birth weights between 1,245 and 2,326 Gm., 25 were given vitamin B_{12} intramuscularly in doses of 10 mcg. the other 23

controls. Frequency of administration varied from daily to every three days the average total dose ranged from 30 to 220 mcg. Treatment was started on the second to seventh day of life.

Results were obtained which indicate that vitamin B_{12} is ineffective in promoting weight gain of normal premature infants. It is suggested that vitamin B_{12} administration will not benefit premature infant unless a specific deficiency of the vitamin exists.

*The Hahnemann Medical College and Hospital
Philadelphia, Pa.*

491 RASCOFF H., DUNEWITZ, A., and NORTON R.: *The weight progress of premature infants given supplementary feedings of vitamin B_{12} a comparative study* J Pediat. 39 61-64, July 1951.

492. MITCHELL, T., ETTELDORF J N., TUTTLE, A. H., and CLAYTON G. W *Crystalline vitamin B_{12} and sodium folvate in prematurity* Pediatrics 8 821-827 Dec. 1951

Crystalline vitamin B_{12} and sodium folvate, separately or together were given intramuscularly to 52 premature babies. Records of weight, length, hemoglobin, hematocrit, erythrocyte count, reticulocyte count, and calculations of the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume failed to show any influence of these vitamins, singly or in combination during the first 50 days of life, on these infants as compared with 24 premature infants who did not receive the vitamins.

*University of Tennessee College of Medicine, and
John Gaston Hospital
Memphis, Tenn.*

493 PARADISO M., and MONDANI E. *Action of vitamin B_{12} on increase of immaturity* Riv clin. pediat. 49 590- 1951 (abstr A.M.A. Am. J Dis. Child. 83 523, April 1952)

"The authors tested the action of vitamin B_{12} in premature infants and noted its therapeutic efficacy thus confirming the results of experimental researches made by others on animals. The authors consider the possible mechanism of action of the vitamin."

494 CHINNOCK, R. F., and ROSENBERG H. W *Results of administration of vitamin B_{12} to newborn infants,* J Pediat. 40 182-185, Feb. 1952.

The effect of oral vitamin B_{12} on weight gain and growth in height was studied in full-term infants. The daily dose of vitamin B_{12} was 5 mcg., and was added to the milk once daily Twelve infants received the vitamin for the first 60 days of life, and 11 received it for 30 days 6 served as controls and were given a placebo. No difference in weight gain or increase in length was noted in infants receiving vitamin B_{12} as compared with the controls.

*College of Medical Evangelists
Los Angeles, Calif.*

ANALYTICAL STUDIES

Vitamin B_{12} Activity in the Body

495. GRANADOS, H., GLAVIND J., NOER, B., and DAM, H.: *Studies on the growth-promoting action of human saliva,* Acta path. et microbiol. Scand. 27 501-505, 1950.

Concentration of vitamin B_{12} in human saliva, determined by a microbiological method, was found to be 0.00015 to 0.0005 micrograms per milliliter

496. TOMASZEWSKI, W *The fluorescence phenomenon of the tongue,* Brit. M. J 1 117 120, Jan. 20, 1951.

When the mouth is viewed under ultraviolet illumination screened with Wood's glass a reddish-orange fluorescence is often visible on the dorsum of the tongue and sometimes on the teeth. This fluorescence is due to the production of porphyrins by oral microorganisms.

The author has examined more than 400 patients for fluorescence of the surface of the tongue and teeth. These patients had various diseases, some characterized by changes in the tongue. More than 200 healthy persons were also examined for control purposes. In some cases, antibiotics, vitamins, and chemotherapeutic agents were given in order to investigate the influence of these substances in the fluorescence.

It was found that fluorescence was absent more frequently with increasing age, being absent in only 11 per cent of children and in 53 per cent of people over 80 years of age. Fluorescence is absent more frequently in certain diseases, such as pernicious anemia, hypochromic anemia, sprue syndrome, and vitamin B deficiencies than in the healthy state. When vitamins of the B group, including vitamin B_{12}, folic acid and riboflavin, are administered, fluorescence is restored and/or intensified in a large percentage of cases. Antibiotics destroy fluorescence by their action on microflora.

The fluorescence phenomenon seems to be dependent on two factors the presence of porphyrin-producing bacteria, and the presence of normal papillae on the tongue. Its clinical significance is not known. It may be a guide to the state of nutrition in the individual.

*Royal Infirmary
Edinburgh, Scotland*

497 ROSS, G. I M. *Vitamin B_{12} assay in body fluids,* Nature 166 270-271 Aug. 12, 1950 (Letter to Editors)

The writer describes a modification of Hutner's technic for the assay of vitamin B_{12} in body fluids, using the alga Euglena gracilis variant bacillaris. This method is more sensitive than the Lactobacillus method.

*Postgraduate Medical School of London
London, England*

498. ROSS, G I. M.: *Vitamin B_{12} assay in body fluids using Euglena gracilis,* J Clin. Path. 5 250- Aug. 1952.

499 ROSENTHAL, H. L., and SARETT H P *The determination of vitamin B_{12} activity in human serum*, J Biol. Chem. 199 433- Nov 1952 (abstr J Am. Dietet. A. 29 162, Feb. 1953)

Preliminary treatment of human serum necessary for microbiologic determination of vitamin B_{12} activity with Lactobacillus leichmannii was studied with samples of serum in the postabsorptive state. The results indicate that the vitamin B_{12}-active material in serum is firmly bound and is not readily released by dialysis but is liberated by heating under suitable conditions."

500. MOLLIN D L., and ROSS G. I. M *Vitamin B_{12} in practice*, Lancet 1 46, Jan. 3, 1953.

The authors state that patients with vitamin B_{12} deficiency can be separated from those with folic acid deficiency by microbiological assay of vitamin B_{12} in the serum, using Englena gracilis var bacillaris. Serum from patients with vitamin B_{12} deficiency shows a low vitamin B_{12} content, usually below 50 micromicrograms per cc. Serum from normal subjects and from patients with folic acid deficiency always has an assay of vitamin B_{12} above 100 micromicrograms per cc.

Occasional false high assays are encountered, but if more than one pre-treatment serum sample is tested, "the method becomes very reliable, and a definitely low serum B_{12} concentration can be confidently accepted as proof of severe vitamin B_{12} deficiency in the body"

Postgraduate Medical School of London
London, England

METABOLIC PROCESSES

501. JUTTON, D R., and PARSONS, H. T *Excretion of vitamin B_{12} by human subjects*, Federation Proc. 10 203, March 1951.

"Intramuscular injection of 450 mg. vitamin B_{12} for 4 subjects (women) led to a urinary return of 37-50% of the dose within 6 hours and 40-75% within 12 hours, in agreement with reports of others on men. In agreement also was the almost complete failure to find vitamin B_{12} activity in the urine on normal diets or after oral dosage. After parenteral administration, a small amount of microbiological activity for *Lactobacillus leichmannii* 4797 (A.T.C.C.) was detected in the samples of bile-rich duodenal contents within 30 minutes, while the basal specimens showed none. Observations on the vitamin B_{12} activity of the feces of these subjects gave no clear evidence of increases following dosage."

University of Wisconsin
Madison, Wis.

502. REGISTER, U D., and SARETT, H. P: *Urinary excretion of vitamin B_{12}, folic acid and citrovorum factor in human subjects on various diets*, Proc. Soc. Exper Biol. & Med. 77 837-839 Aug. 1951.

Recent reports have suggested that the urine of normal subjects has no appreciable vitamin B_{12} activity. In the present study small but measurable amounts of vitamin B_{12} were found in the urines of 56 medical students on various diets. In 14 subjects on normal diets, the urinary excretion of vitamin B_{12} averaged 31 mγ (millimicrogram) a day Similar values were found in 28 subjects maintained for three days on milk, alkaline ash, low protein, or high protein diets. A somewhat higher value was found in 8 subjects on high purine diets, and the increase over normal was shown to be due to interfering compounds. On the third days of fasting, 6 subjects were found to excrete an average of 62 mγ of vitamin B_{12} a day The average citrovorum factor (CF) excretion by the subjects on these diets accounted for only part of the total folic acid (FA) compounds excreted and appeared to be correlated with the total FA values. Differences in vitamin B_{12} excretion resulting from dietary changes where not similar to those observed in CF and FA excretion.

Tulane University School of Medicine
New Orleans, La.

503. LANG C., HARTE, R. A., CONLEY C. L., and CHOW B. F *Retention of crystalline vitamin B_{12} by healthy male individuals following intramuscular injection*, J Nutrition 46 215-221 Feb. 11, 1952.

Authors summary "Crystalline vitamin B_{12} was administered intramuscularly to groups of healthy male subjects at the following levels 20 30, 50, and 75 μg. Urinary collections from each subject were made during 24 hours after injection, each in two fractions represent ing the first 8 hours and the following 16 hours. The vitamin B_{12} microbiological activities in the urine speci mens were measured. These data permitted calculation of the amount retained, by taking the difference between the injected and the excreted vitamin. It was found that more than 50 μg. of vitamin were retained daily by these subjects, depending on the dosage administered."

The Johns Hopkins Hospital
Baltimore, Md.
Sharp and Dohme Inc.
Glenolden, Pa.

504 WATKIN D M., LANG C. A., SHOCK, N W., and CHOW B. F *Age-wise differences in urinary excretion of vitamin B_{12} following intramuscular administration*, Federation Proc. 12 151 March 1953.

"The urinary excretion of vitamin B_{12} in 24 hours following its intramuscular administration in crystalline form was studied at different dose levels in 107 male subjects in widely separated age categories. The results of two separate studies [summarized in tabular form] demonstrate that B_{12} excretion assayed microbiologically diminished in an orderly and significant fashion with age. Significance was tested at the 0.05 level by an analysis of variance. Decrements in B_{12} excretion with age parallel previously observed decrements in renal function with age. However the observed differences cannot, on the basis of data arising from these studies, be attributed to renal factors alone."

National Heart Institute
Bethesda, Md.
Baltimore City Hospitals, and
Johns Hopkins School of Hygiene and Public Health
Baltimore, Md.

505. LANG C. A., BECKER, B., GLEYSTEEN D., and CHOW B. F: *Diabetic retinopathy and urinary excretion of vitamin B_{12}*, Federation Proc. 12 421, March 1953.

"The marked retention of radioactivity by the pancreas following injection of radiovitamin B_{12} and its involvement in carbohydrate metabolism suggest a possible role of vitamin B_{12} in pancreatic diseases such as diabetes mellitus, which might manifest itself in an abnormal excretion of a test dose. The results of an initial experiment indicated a correlation in the urinary excretion of administered B_{12} and diabetic retinopathy. To examine this hypothesis, consecutive diabetic patients seen at The Wilmer Institute regardless of age, sex, or other diseases were injected intramuscularly with 50 mcg. crystalline vitamin B_{12}. These patients were classified as to the presence or absence of retinopathy. Urine specimens collected prior to and from 0-8 hours after administration were assayed for the total microbial activity of B_{12}. To avoid bias the diagnoses and B_{12} analyses were not correlated until the final assay results were obtained. In this series of 35 diabetics the mean urinary excretions of B_{12} were 4.2 $\pm$ 1.7 mcg. for the 13 subjects without retinopathy and 19 $\pm$ 2.1 mcg. for the 22 subjects with retinopathy. The latter value was statistically higher than that (9.6 $\pm$ 1.4) of healthy subjects in a comparable age range. Only two individuals, both in the non retinopathy group excreted amounts overlapping into the opposing group. Although subsequent examination disclosed the presence of retinal disease, they were not removed from their original group for statistical analyses. Even then a significant difference ($P << 0.001$) between the two diabetic groups was obtained. The lower B_{12} activity in the urine could be due to the excretion of metabolically inactivated B_{12}, the presence of inhibitors, or a greater retention of the vitamin. However when B_{12} containing radioactive Co^{60} was injected into several subjects in both diabetic groups, a direct correlation of radioactivity and microbial activity was found in the urine. These results thus favor the last hypothesis."

The Johns Hopkins University
Baltimore, Md.

506. UNGLAUB, W. G., ROSENTHAL, H. L., and GOLDSMITH, G. A. *Vitamin B_{12} in blood and urine after oral and parenteral administration*, Federation Proc. 12: 432, March 1953.

"Following intramuscular administration of 10 to 100 µg. of vitamin B_{12} to normal subjects, serum vitamin B_{12} activity rose in one hour to maximum values which were proportional to the amounts injected. Urinary vitamin B_{12} activity in 24 hr. following injection increases with the quantity administered, but cannot be predicted accurately. Serum vitamin B_{12} activity during fasting is consistently lower in patients with untreated macrocytic anemia than in normal subjects. In 2 patients with pernicious anemia in relapse who received 25 µg. of vitamin B_{12} intramuscularly increases in serum activity did not differ from those of normal subjects but vitamin B_{12} activity in urine was somewhat less than normal. Serum vitamin B_{12} acitivity did not increase significantly when normal subjects were given 500 and 1000 µg. orally. After oral administration of 3000 µg., serum activity reached maximum values equal to those found after intramuscular injection of 10-25 µg. Serum activity remained elevated for longer periods after oral than after intramuscular administration. In 3 of 4 patients with macrocytic anemia who received 3000 µg. of vitamin B_{12}, orally serum activity increased to maximum value similar to those noted after intramuscular injection of 1-50 µg. In the 4th no significant rise occurred. In the 5th patient serum activity increased after oral administration of 500 and 1000 µg. Optimal hematologic response resulted in all patients. Following oral doses, no significant increase in vitamin B_{12} activity was found in the urine."

Tulane University School of Medicine
New Orleans, La.

507. STUHLFAUTH, K. *Influence of vitamin B_{12} on levulose content of blood*, Klin. Wchnschr. 31: 312, April 1, 1953 (abstr. J.A.M.A. 152: 1382-1383, Aug. 1, 1953)

"Steinitz reported in 1937 that the increased blood levulose concentration that results from the administration of levulose can be reduced by administration of liver extract. Stuhlfauth noted that the same effect could be obtained when, instead of liver extract, he administered from 15 to 30 µg. of vitamin B_{12} intramuscularly after levulose had been given either intravenously or by intraduodenal drip. Stuhlfauth was interested in ascertaining whether this reduction in the blood levulose content, which he had obtained in 30 subjects, including some with hepatic disorders and with obesity, would also influence other constituents of the carbohydrate metabolism. For this purpose levulose was introduced into the duodenum of 10 fasting persons. When 1 liter of 5% levulose solution was given hourly, the blood levulose concentration was found to be between 30 and 50 mg. per 100 cc. after about 120 minutes and remained at this level when the levulose administration was continued at the same rate. ... If, after the levulose content has reached its maximum ... the patient is given 30 µg. of vitamin B_{12}, the levulose nearly always decreases by from 10 to 25 mg. per 100 cc. The blood dextrose then increases slightly ... Lactic acid usually increases noticeably, sometimes to twice its original value, one hour after injection of vitamin B_{12}. The pyroracemic acid decreases following a slight temporary increase. The inorganic phosphate one hour following the injection of vitamin B_{12} shows a renewed increase, which is followed by a temporary decrease. The reduction in the blood levulose content occurs only after injection of vitamin B_{12} or of liver extracts. Cobalt, folic acid, and adrenal cortex extract did not have this effect on the levulose content of the blood. Administration of 30 µg. of vitamin B_{12} to eight fasting healthy persons who had not been given levulose likewise showed a slight decrease in the general function of reduction. In five patients with diabetes no uniform changes could be observed. The author concludes that the changes appearing simultaneously in the serum contents of dextrose, pyroracemic acid, and inorganic phosphate indicate that vitamin B_{12} induces increased utilization of levulose."

Nutrition in General

508. KING, C. G. *New advances in the science of nutrition*, J. Am. Dietet. A. 25: 109-111, Feb. 1949

Nutrition Foundation, Inc.
New York, N. Y.

509. SPIES, T. D.: *Recent progress in nutrition*, Post grad. Med. 6: 96-106, Aug. 1949

A *number of subjects* are briefly discussed in this paper One emaciated patient with nutritional macrocytic anemia responded well to folic acid. Charts and photographs show his improvement under adequate treatment. A patient with sprue also made a good response to folic acid. Lateral sclerosis in pernicious anemia, however is *not prevented or relieved* by folic acid. In this condition vitamin B_{12} is effective. When given intramuscularly only very small doses are required: 8 micrograms in tropical and non-tropical sprue, and 15 micrograms in pernicious anemia. Intravenous administration has been used in one series of patients and subcutaneous in another *and both methods have proved* effective and non toxic. When vitamin B_{12} is given orally about 50 times as much is required to produce a given blood response as when it is given intramuscularly The oral dose can be reduced to one-fifth or one-tenth, however if the vitamin *is incubated overnight with normal human gastric juice.*

Similar results have been obtained with vitamin B_{12} in nutritional macrocytic anemia and in pernicious anemia. In 2 patients, symptoms of very acute combined system disease disappeared following the oral administration of 1.5 mg. of vitamin B_{12} over a period of 10 days. Massive doses are necessary in this condition, but the results depend more on the chronicity of the disease than on the extent of the neurologic involvement. The most dramatic responses have occurred in patients with symptoms of less than a month s duration.

Hillman Hospital
Birmingham, Ala.

510. HAUSMANN K. *Liver extracts, vitamin B_{12}, and thymidine*, Lancet 2 962-963, Nov 19 1949 (in Letters to the Editor)

The writer assumes the possible existence of at least three groups of different forms of vitamin B_{12} 1) cobalt containing red compounds which on intramuscular injection, even in high doses, are ineffective in pernicious anemia but which with enzymatic cleavage become hema topoietically active 2) cobalt-containing red pigments that are clinically active and precipitable by *ammonium* sulfate, as contained in purified liver extracts and described by Lester Smith 3) cobalt-containing red pig ments, clinically active but soluble in ammonium sulfate, which are present especially in autolyzed liver and in urine. It is suggested that under the influence of certain enzymes, which the writer proposes to designate as vita min B_{12} conjugases and which are to be found in stomach mucosa, pancreas, kidneys, and presumably also in liver papain, moulds, and bacteria, the different forms of vita min B_{12} may become transformed one into the other and vary in the liver according to the degree of autolysis.

St Georg General Hospital
Hamburg, Germany

511 MUSHETT C. W *Vitamin B_{12}—A nutritional factor for man and beast*, Am. Biol. Teacher 12 134-141, Oct. 1950.

512. SPIES T D *Recent advances in diagnosis and treatment of deficiency diseases*, J.A.M.A. 145 66-72, Jan. 13, 1951.

Difficulties in diagnosing conditions of undernutrition and malnutrition are discussed and illustrative case histories are presented.

The author details his current method of giving vita mins orally to patients with signs of nutritional deficiency (including the clinical syndromes of sprue, beriberi, pellagra, *riboflavin deficiency scurvy and certain macrocytic* anemias) The following daily doses of vitamins are given folic acid, 5 mg. thiamine, 10 mg nicotinamide, 150 mg. riboflavin, 10 mg. ascorbic acid, 150 mg. and vitamin B_{12}, 10 mcg.

Experiences with folic acid and vitamin B_{12} in the treatment of anemias are reviewed. Dosage of vitamin B_{12}, both oral and parenteral, is discussed. The minimum effective parenteral dose is 1 mcg. daily and the effective oral dose in most patients is 30 to 60 times the parenteral dose. *The author's studies to date show no demonstrable difference in activity per unit of weight for* vitamin B_{12}, vitamin B_{12a} and vitamin B_{12b}, the last two being identical substances. It is stated that concentrates of vitamin B_{12} seem to be about as well tolerated as solutions [of the crystalline preparation?]

Northwestern University Medical School
Chicago, Ill.

513 REVIEW: *Festival symposium on nutrition and the B vitamins*, Brit. M. J 2 49-51 July 7 1951.

This is a report of the speeches made at the symposium. Subjects discussed were vitamin B_1 deficiency in man, nature and occurrence of vitamin B_{12}, treatment of megaloblastic anemia with vitamin B_{12}, malnutrition in West Africa, and pellagra.

514 GOLDSMITH, G A., and GIBBENS J: *Recent advances in nutrition review of the literature* 1949-1950, A.M.A. Arch. Int. Med. 88 93-131 July 1951.

In the section of this review devoted to vitamins, subjects discussed are folic acid, citrovorum factor vita min B_{12} and choline in liver disease; vitamin A, folic acid, vitamin B_{12}, choline, inositol, thiamine, riboflavin, pyridoxine, vitamin B complex, ascorbic acid, vitamin D vitamin E, and vitamin K.

Tulane University School of Medicine
New Orleans, La.

515 SPIES T D., STONE, R. E., GARCIA LOPEZ, G., LOPEZ TOCA, R., and REBOREDO A. *Therapeutic indications for vitamins in mixtures* Post grad. Med. 10 269-284 Oct. 1951.

A patient who had a low plasma ascorbic acid level developed areas of hemorrhage around hair follicles on the leg. The hemorrhagic areas faded within 72 hours after the daily administration of 500 mg. of synthetic ascorbic acid was begun. (Rats fed on the diet used by the patient did poorly Supplementation with the B vita mins improved their condition, and liver extract was still more effective.) *It was expected that the patient would* develop symptoms of other deficiency diseases, and event ually *she did develop a* macrocytic anemia. Administration of folic acid resulted in striking improvement. Glossitis developed and was relieved by vitamin B_{12}, 1-

a day Later edema developed and a diagnosis of beriberi was considered. This condition was relieved by thiamine treatment.

Vitamin B_{12} was effective in a case of acute combined degeneration of the spinal cord. A patient with pernicious anemia responded at different times to a number of agents—folic acid, 5-methyl uracil, vitamin B_{12}, vitamin B_{12a}, and B_{12b}, and folinic acid. A patient with pellagra responded to folic acid, and later her nursing child was successfully treated for anemia with folic acid followed by a single injection of vitamin B_{12}. A patient with macrocytic anemia of pregnancy failed to respond to vitamin B_{12} intramuscularly or orally but responded to oral administration of vitamin B_{12} combined with folic acid. The diarrhea associated with tropical sprue may be relieved by either vitamin B_{12} or folic acid, and sometimes (but rarely) the combination of the two is more effective than either alone. Illustrations are presented of a child with cheilosis due to riboflavin deficiency and one with rickets.

Dietaries of families with nutritional deficiency diseases are discussed, and dietary and basic therapy are outlined. Methods of administration of the vitamins are discussed. Basic vitamin therapy includes thiamine, riboflavin, niacinamide, ascorbic acid, folic acid orally and vitamin B_{12} when it can be absorbed. In addition, synthetic vitamins may be given orally or parenterally and natural B complex in the form of brewer's yeast or extract and/or liver extract may be given orally or parenterally

Northwestern University Medical School
Chicago, Ill.
Hillman Hospital
Birmingham, Ala.
General Calixto Garcia Hospital
Havana, Cuba

516. TOMARELLI, R. M., LINDEN E., and BERNHART F W: *Nutritional quality of milk thermally modified to reduce allergic reaction*, Pediatrics 9 89-93, Jan. 1952.

Infants and children allergic to cow's milk, can take milk which has been made hypoallergenic by prolonged heat treatment, but experiments with rats have shown that milk thus treated is reduced in nutritional quality Fortification of heat-treated milk with minerals and all the usual vitamins with the exception of vitamin B_{12} did not restore its nutritional value for young rats, but addition of liver powder or of a vitamin mixture containing vitamin B_{12} resulted in a growth rate equal to that of rats fed unheated milk. The addition of aureomycin to the heat-treated milk, with or without vitamin B_{12}, did not increase the growth rate.

Wyeth Incorporated
Mason, Mich.

517 CHOW B. F: *The role of vitamin B_{12} in metabolism*, South. M. J 45 604-612, July 1952.

Pharmacologic and metabolic studies in the literature are reviewed, and the author gives the results of his own investigations. Urinary and fecal excretion of vitamin B_{12} and the content of the vitamin in kidney liver and intestines after administration were studied. Nitrogen retention in infants appeared not to be affected by vitamin B_{12} supplementation. Children with chronic diseases (stress) appeared to gain more weight and to gain it more rapidly when they were given 25 mcg. of vitamin B_{12} daily by mouth in healthy children the results of such supplementation were equivocal. Vitamin B_{12} appears to play no direct part in protein metabolism but it is apparently involved in carbohydrate and/or fat metabolism. Its effect on weight gain in young growing female rats is greater than in littermate males when the major source of calories is carbohydrate, and is greater when the diet is high in carbohydrate than when it is high in fat or in fat-carbohydrate mixtures.

Johns Hopkins University
Baltimore, Md.

518. SURE, B.: *Improvement in protein efficiency of a whole wheat cereal breakfast food with the amino acids lysine and threonine and vitamin B_{12}*, Arch. Pediat. 69 359- Sept. 1952 (abstr J Am. Dietet. A. 29 56, Jan. 1953)

"Enrichment of cereal grains promises a more nutritious diet for large portions of the world's population who cannot afford to supplement their diets with a sufficient quantity of the important animal products, and for whom cereals are the basic food. An investigation of the value of the protein enrichment of cereals was made on albino rats. The rations consisted of a processed whole wheat cereal breakfast food plus supplements of vitamin B_{12}, lysine, valine, threonine, and a fish-soluble extract in various combinations. The B_{12} supplement proved of no value in increasing growth, but when combined with the fish-soluble extract or with the three amino acids resulted in remarkable growth."

519 GOLDSMITH, G A., UNGLAUB, W G., and GIBBENS, J *Recent advances in nutrition and metabolism review of the literature, 1951* A.M.A. Arch. Int. Med. 90 518-561, Oct. 1952.

This review covers 350 references, most of them multiple, to nutrition and metabolism. The subjects reviewed include caloric nutrition, fat metabolism, nutrition and hypertension, protein nutrition, carbohydrate nutrition, vitamins (A, thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid, citrovorum factor B_{12}, choline, inositol, ascorbic acid, D E, and K) and mineral nutrition. Sprue, and nutrition in liver disease are discussed.

Tulane University School of Medicine
New Orleans, La.

520. COWGILL, G R. *Recent trends in nutrition and future possibilities*, Connecticut M. J 17 91-97 Feb. 1953.

Nutritional studies during the first half of the century have shown that the energy or caloric factor the protein factor certain mineral or inorganic nutrients and vitamins are essential.

There is some evidence that the caloric requirements calculated from surveys made at the turn of the century are now too high since muscular activity has decreased during this period.

Study of protein has shown that 10 amino acids are essential for the growth of the rat, and 8 for maintenance

of nitrogen equilibrium in man. It is now recognized that proteins are as important nutritionally as vitamins.

Many antivitamins have been discovered which can replace the natural vitamin for the first steps in a sequence of chemical changes but cannot complete the sequence. This may be advantageous if it interferes with the growth of disease bacteria which depend on a certain vitamin, or it may produce symptoms of vitamin deficiency. Recently pyridoxine deficiency has been produced for the first time in human volunteers by the use of the antivitamin desoxy pyridoxine, showing that pyridoxine is essential for man.

The history of the development of vitamin B_{12} and its effect in pernicious anemia and in promoting growth are discussed.

Studies in progress at the Institute of Nutrition of Central America and Panama (INCAP) suggest that humans require a small amount of animal protein and that supplements of B_{12} and/or certain antibiotics (penicillin, aureomycin) may promote growth in children who receive little or no animal protein, and may improve plant food dietaries.

Low sodium diets are discussed and the danger of too great depletion of other minerals, protein and B-complex vitamins by means of such diets is pointed out. Informative labeling of food products processed to reduce sodium content is urged.

Another topic discussed is the effect of fats and cholesterol on hardening of the arteries.

Methods of improving nutrition by improving the quality of foods include the enrichment of wheat flour of rice (to prevent beriberi) and of corn meal (to prevent pellagra). Another means of preventing pellagra is to encourage the use of milk, since milk contains tryptophan and this amino acid is used by the body in manufacturing nicotinic acid, the antipellagra vitamin.

Quality of foods might further be improved by genetic selection of varieties of plants which have high content of vitamins (thiamine, niacin, ascorbic acid).

Yale University
New Haven, Conn.

521. ELVEHJEM, C. A.: *Value of natural foods*, J. Am. Dietet. A. 29: 178, Feb. 1953.

In an address before the Nutrition Foundation, the following reference to vitamin B_{12} was included.

"Since we now know that most of the vitamins function by supplying the prosthetic group or the working end of an enzyme, it is not surprising to find some relation between amino acids needed for building protein and a vitamin. Recently it has been found that vitamin B_{12} protects the young rat on a diet low in choline and methionine against a fatty liver and also against kidney damage. Work in our laboratory using a low-protein diet of 9 per cent casein has shown that in the absence of choline, B_{12} has little, if any protective action on the production of fatty livers. This undoubtedly means that with no choline and a low supply of methionine, vitamin B_{12} is unable to overcome any of the deficiency. When small limiting levels of choline and a little higher levels of methionine are used, the vitamin B_{12} does have some sparing effect. We have recently found that even in the presence of adequate methionine, choline, and vitamin B_{12}, the fat content of livers is not normal when a low protein diet is fed. Certain other amino acids are needed to bring the fat content of the liver to a normal level. Furthermore, some of these changes can be aggravated by an imbalance of certain amino acids. This emphasizes the importance of supplying amino acids in foods rather than in combinations which might be quite different from those usually encountered in food materials."

University of Wisconsin
Madison, Wis.

522. MICKELSEN, O.: *Nutritional aspects of antibiotics*, J. Am. Dietet. A. 29: 221-229, March 1953.

The literature on the increase in growth of animals when antibiotics are added to commercial rations is reviewed. Whether the increase is the result of a sparing action on vitamin and protein requirements, favoring the growth of microorganisms which synthesize some unknown growth factor, decreasing the growth of bacteria which would use large amounts of some essential dietary nutrient, inhibition of the growth of microorganisms which might produce disease, or favoring the absorption of nutrients from the gastrointestinal tract is not yet known. The increase in the rate of growth of animals may lead to cheaper and more abundant supplies of meat for human consumption.

Aureomycin has been found to produce improvement in clinical cases of pernicious anemia, perhaps by lessening the patient's vitamin B_{12} requirement, probably by increasing the synthesis of this vitamin in the gastrointestinal tract. Improvement of megaloblastic anemia of pregnancy under penicillin treatment has also been reported. Perrini (*Boll. Soc. ital. biol. sper.* 27: 1151, 1951; *Chem. Abstr.* 46: 5192, 1951) has reported increase in weight gain in infants given aureomycin, as has Robinson (*Lancet* 1: 52, 1952).

Studies are being conducted by the Institute of Nutrition of Central America and Panama (*Proc. Food and Nutrition Board* 12: 21, May 1952) on the effect of supplementing the diets of school children with skim milk, vitamin B_{12} (20 mcg. a day) or aureomycin (50 mg. a day). In children receiving only 5 Gm. of animal protein a day skim milk slightly increased growth, vitamin B_{12} was considerably more effective, and aureomycin produced a growth rate almost twice that of children receiving placebos. In children receiving 13 Gm. of animal protein a day neither vitamin B_{12} nor aureomycin increased the growth rate. The possibility of improving, by the addition of B_{12} or antibiotics to the diet, the nutritional status of the peoples who receive little animal protein is suggested. In this country antibiotic supplementation would probably not be dramatic since the usual intake of animal protein is fairly high.

National Institutes of Health
Bethesda, Md.

523. SEBRELL, W. H., JR.: *Trends and needs in nutrition*, J.A.M.A. 152: 42-44, May 2, 1953.

In this discussion of the present status and the future of nutrition as it relates to the field of medicine, the following excerpts deal with the place of vitamin B

the total nutritional picture " interrelations have been established between choline and the vitamins B_{12}, folic acid, and ascorbic acid and these have been related to the amino acids cystine, methionine, tyrosine, and phenylalanine.

"In other studies continued attempts have been made to clarify the mechanism through which a lack of choline may damage the kidneys and liver Absence of this vitamin from the diet has killed rats within five days. The specific effect, apparently vascular can be minimized by several procedures, including administration of vitamin B_{12}, corticotropin, or nonspecific stimuli including an alarm reaction "

In summary the author states: "A reoriented nutrition program in this country is much needed, with increased emphasis on clinical research aimed primarily at obesity chronic diseases, and borderline deficiencies. In addition, there is need for intensified research on the special nutritional requirements of the aged and persons under stress."

National Institutes of Health
Bethesda, Md.

ANIMAL STUDIES

Fertility Studies

Chicks

524 LILLIE, R. J., OLSEN M. W., and BIRD H. R.: *Role of vitamin B_{12} in reproduction of poultry* Proc. Soc. Exper Biol. & Med. 72 598-602, Dec. 1949

During the incubation period eggs laid by Rhode Island Red hens fed a diet deficient in vitamin B_{12} were injected with 0.5 to 1.25 mcg. of vitamin B_{12} per egg in aqueous solution. The rate of hatchability of these eggs was greater than that of eggs injected with sterile water or not injected. In the chicks from the eggs injected with vitamin B_{12} the mortality was lower the growth rate was greater and the feathering better than in chicks hatched from eggs injected with water or not injected. Adding vitamin B_{12} to the diet of chicks hatched from vitamin B_{12} deficient eggs increased the growth response but did not reduce the mortality nor improve the feathering. This emphasizes the importance of the maternal diet, which, if deficient, may lead to irretrievable postnatal damage as well as prenatal effects. The greater growth response occurred from a combination of dietary supplement with egg injection.

U. S. Department of Agriculture
Beltsville, Md.

525. OLCESE, O., COUCH, J R., and LYMAN C. M. *Vitamin B_{12} concentrates in the nutrition of the mature domestic fowl*, J Nutrition 41 73-87 May 1950.

Authors summary "When a sucrose-soybean protein diet, low in vitamin B_{12}, was fed to a group of hens, egg production decreased. Hatchability of eggs from hens fed this low vitamin B_{12} diet decreased to zero in from 8 to 6 weeks. The addition of APF concentrates improved the egg production and hatchability of hens fed the purified diet but failed to produce normal hatchability as compared to a group fed a practical all-mash diet. When a starch-soybean protein diet was used, egg production and hatchability were improved over those observed with comparable groups fed sucrose as a source of carbohydrate, indicating that starch prompted the intestinal synthesis of vitamin B_{12} and possibly some unknown factor required for embryonic development in the fowl. The hatchability of eggs from hens receiving the purified diets showed a tendency to decrease about the 9th or 10th experimental week, even though adequate amounts of vitamin B_{12} were fed. This indicates that a depletion of some unknown factor or factors may have taken place after the initial depletion of vitamin B_{12}."

Agricultural and Mechanical College of Texas
College Station, Texas

526. OLCESE, O., COUCH, J R., QUISENBERRY H. H., and PEARSON P B.: *Congenital anomalies in the chick due to vitamin B_{12} deficiency* J Nutrition 41 423-431, July 1950.

A definite peak of embryonic mortality occurred at the 17th day of incubation in eggs from hens fed a diet deficient in vitamin B_{12}. The most characteristic symptom of deficiency present in the embryos was myoatrophy of the leg. A malposition, "head between the thighs," occurred with unusual frequency in eggs from hens fed a vitamin B_{12}-deficient diet. Other anomalies associated with vitamin B_{12} deficiency in this study were hemorrhages of the embryo and allantois, and perosis.

527 KLINE, I. T., and DORFMAN R. I. *Estrogen stimulation of the oviduct in vitamin-deficient chicks*, Endocrinology 48 345-357 April 1951.

The relation of single vitamin deficiencies to the action of stilbestrol on the oviduct of the chick was studied. The inanition which accompanies a state of vitamin deficiency is responsible for a nonspecific, reduced sensitivity of the oviduct to the action of estrogen. When this reduced sensitivity is taken into consideration it is found that estrogen exerts a greater than-expected stimulation in thiamine-deficient pullets, probably explainable on the basis of greater availability of estrogen because of failure of inactivating mechanisms.

In riboflavin-deficient pullets the reduced response to estrogen may be accounted for superficially at least, on the basis of inanition alone but may be the algebraic sum of numerous factors such as reduced inactivation of estrogen and decreased functioning of the mechanisms for tissue proliferation. In nicotinic acid-deficient pullets low doses of estrogen can exert a greater-than-expected influence on oviduct growth but high doses are less effective than in inanition controls. A deficiency of folic acid however results in a severe limitation of response to estrogen at all dose levels. The implication is that folic acid is essential to the mechanism of action of estrogen in promoting tissue proliferation in the chick oviduct at the level of the end organ itself.

The relationship of vitamin B_{12} to stilbestrol action could not be determined on the basis of the data obtained *in this experiment. It was found,* however that there is significantly greater response to 3.2 mg. dose of stilbestrol in Sex-linked Cross breed on commerical mash when it is supplemented with vitamin B_{12}, but that supplementation of a synthetic diet with vitamin B_{12} significantly decreases the oviduct response to this dose of stilbestrol.

Western Reserve University School of Medicine, and Lakeside Hospital Cleveland, Ohio

528. MARIAKULANDIA, A., MYINT T., and Mc GINNIS, J *Effect of terramycin and vitamin B_{12} on hatchability* Proc. Soc. Exper Biol. & Med. 79 242-244, Feb. 1952.

White Leghorn pullets, 8 weeks old, were divided into four groups of approximately 90 birds each. The pullets were kept on range and fed an all-plant protein ration supplemented with 12.5 mcg. of B_{12} per pound of feed. Two groups also received terramycin at a level of 15 ppm.

At 22 weeks of age B_{12} supplementation was withdrawn from one of the antibiotic fortified groups and from one group receiving the B_{12} alone. The birds were then housed in individual cages with wire floors and continued on experiment for a period of 10 months.

The vitamin B_{12} and terramycin supplements alone and in combination had little or no effect on egg production and gain in weight. In the absence of B_{12}, hatchability of fertile eggs was definitely improved by feeding terramycin. There was an indication that terramycin fed in combination with vitamin B_{12} further improved hatchability

No great increase in the B_{12} content of eggs from the terramycin-supplemented lots was observed.

State College of Washington Pullman, Wash.

Mice

529 JAFFÉ, W G. *Reproduction of mice kept on rations low in vitamin B_{12}.* Arch. Biochem. 27 464-466, July 1950.

530. JAFFÉ, W G *Influence of cobalt on reproduction of mice and rats,* Science 115 265-267 March 7 1952.

Experiments with 130 litters of mice and 64 litters of rats show that cobalt influences the number of weaned young per litter Cobalt was administered as $CoCl_2$ or in vitamin B_{12}. $CoCl_2$ was given parenterally in drinking water or in food. While the vitamin B_{12} treated litters had the most weaned young, the animals who received cobalt chloride did almost as well. It is suggested that populations which subsist on diets poor in animal products, and therefore probably poor in vitamin B_{12}, might profit from cobalt supplements.

Instituto Nacional de Nutricion Caracas, Venezuela

Rats

531 SURE, B. *Vitamin B_{12} and folic acid for reproduction and lactation,* Federation Proc. 9 372, March 1950.

"This work was carried out on the Wistar Strain albino rat. A ration containing 50% low-fat soybean flour furnished 25% proteins in rations which were adequate in all dietary essentials except folic acid and vitamin B_{12}. *Ration A* was the control without folic acid and vitamin B_{12} supplementation *ration B* was supplemented with vitamin B_{12}; *ration C* was fortified with folic acid, and *ration D* was supplemented with folic acid and vitamin B_{12}. During a reproduction period of about 4 months the results obtained made it apparent that for optimum reproduction and lactation both folic acid and vitamin B_{12} are essential and that folic acid plays a significant role in fecundity Male rats from mothers on *ration B* weighed after 8 weeks growth, 109 gm. more, and female rats weighed 46 gm. more than rats from mothers on *ration A*"

University of Arkansas Fayetteville, Ark.

532. LEPKOVSKY S., BORSON H. J., BOUTHILET R., PENCHARZ, R., SINGMAN D., DIMICK, M. K., and ROBBINS, R. *Reproduction in vitamin B_{12}-deficient rats with emphasis upon intrauterine injury* Am. J Physiol. 165 79-86, April 1951

Experiments with female rats show that a vitamin B_{12} deficiency causes them to have progressively severe reproductive abnormalities with successive litters. Third litter young that are born are so debilitated as a result of prenatal injury that they do not survive even when nursed by normal females on stock diet. Vitamin B_{12} administered parenterally at birth enables many but not all of these third litter rats to survive. Rats that are weaned on vitamin B_{12}-deficient diets undergo a period of high mortality two to three weeks after weaning, generally dying with a severe leukopenia. In general it *was found that while lactation was impaired by a vitamin* B_{12} deficiency ability to produce live young was even more impaired, only five live litters resulting from 19 positive matings for a third litter

University of California School of Medicine Berkeley and San Francisco, Calif

533. SURE, B. *Vitamin B_{12} in growth, reproduction and lactation,* Federation Proc. 10 395, March 1951.

"Results on the influence of the addition of 0.1 μg. daily of crystalline vitamin B_{12} on growth of the albino rat, during 10 weeks of the post-lactation period, are as follows As a supplement to a ration containing 25% proteins furnished as low fat soybean flour: 15 males, 20% increased growth; 15 females, 7.2% increased growth. As a supplement to a ration containing 28.2% alpha-soybean protein 18 animals, 38.5% gain in body weight. As a supplement to 25% proteins in ration furnished as low fat cottonseed flour: 18 animals, 38% increase in body weight. As a supplement to a ration containing 94% as the breakfast food, Cerevim, the addition of 0.1 μg. daily of vitamin B_{12} for growth, 0.2 μg. for reproduction, and 0.5 μg. for lactation, resulted in marked improvement in reproduction, and lactation efficiency was increased from 37 to 73% Three generations of animals with excellent growth records were obtained when such vitamin B_{12}-supplemented ration furnished all the B vitamins proteins and minerals. The addition of 1% APF fro

fermentation product of aureomycin to the Cerevim ration also produced very favorable effects on reproduction and lactation, and 3 generations of healthy growing animals have been obtained on the latter ration."

University of Arkansas
Fayetteville, Ark.

534 SURE, B.: *Vitamin B_{12} in reproduction and lactation*, J Am. Dietet. A. 27 554-567 July 1951.

Author's summary: "A diet, the proteins of which were furnished by 50 per cent low-fat soybean flour introducing 25 per cent proteins in the ration and satisfactory in all dietary essentials for good growth of the Wistar strain albino rat, was only partially successful for reproduction and lactation in the first generation. In the second generation, this diet was a marked failure for reproduction and a complete failure for lactation. The supplementation of this basal diet with folic acid resulted in a remarkable improvement in reproduction, but lactation efficiency was only 25 per cent. The addition of vitamin B_{12} in minute amounts produced a marked increase in lactation efficiency which was 80 per cent. Vitamin B_{12} also had a favorable effect on reproduction. The addition of both vitamin B_{12} and folic acid produced the optimum results in both reproduction and lactation of first generation animals. Second and third generation animals were successfully reared on this ration supplemented with B_{12} only Fourth generation animals grew well on this diet.

"Excellent results in lactation have been obtained through the second generation with a diet, the proteins of which have been derived from 60 per cent soybean flour supplemented with vitamin B_{12}. This ration unsupplemented with vitamin B_{12} proved a failure for reproduction and lactation."

University of Arkansas
Fayetteville, Ark.

535. SURE, B. *Further studies of vitamin B_{12} in reproduction and lactation*, Arch. Pediat. 68 540-543, Nov 1951.

The effect of vitamin B_{12} on reproduction and lactation was studied in rats. The rats were maintained on a basal ration which already contained a good source of vitamin B_{12} for growth (nonfat milk solids) and to which vitamin supplements (except vitamin B_{12}) were added. The control group received the basal ration plus the vitamin supplements. Another group (3 males and 15 females) were given in addition daily per animal 0.1 µg. of vitamin B_{12} during growth, 0.2 µg. during reproduction, and 0.5 µg. during lactation periods. In a third group the influence of the addition of APF was studied and in a fourth, some of the basal ration was replaced by dry skim milk. Vitamin B_{12} significantly reduced the number of stillbirths and increased lactation efficiency by 30.1 per cent in the first generation. In the second generation the further supplementation of the basal ration with folic acid resulted in 100 per cent lactation efficiency Replacement of some of the basal ration (Cerevim) with dry skim milk resulted in an excellent lactation, in the absence of added B_{12}. Replacement of 1 per cent of the basal ration with an APF product produced excellent lactation efficiency and must have replaced the increased needs of vitamin B_{12} for lactation in the first generation. However there was reduced lactation efficiency in the second generation, even in the presence of folic acid supplementation.

University of Arkansas
Fayetteville, Ark.

536. DRYDEN, L. P., HARTMAN A. M., and CARY C. A. *The relation of vitamin B_{12} deficiency to fertility of the female and birth weight of the young in rats fed purified casein rations*, J Nutrition 45 377-391, Nov 1951.

A vitamin B_{12}-deficient diet did not effect a greater percentage of nonpregnancies or resorptions in rats than there were in controls on a standard diet, but the litters were smaller and average birth weights lower B_{12} supplements improved birth weights, but not litter size.

537 MEYER, M. L., THOMPSON H. T., and ELVEHJEM, C. A. *Effect of vitamin B_{12} on reproduction and lactation in rats receiving pork or beef diets*, J Nutrition 45 551-565 Dec. 1951.

Nutrition and Growth

VITAMIN B_{12}; ANIMAL PROTEIN FACTOR

Chicks

538. OTT W H., RICKES E. L., and WOOD T R.: *Activity of crystalline vitamin B_{12} for chick growth*, J Biol. Chem. 174 1047 1048, July 1948 (Letter to Editor)

Crystalline vitamin B_{12}, when added to purified basal diets low in the "animal protein factor" (unidentified chick growth factor) stimulated the growth of chicks as much as or more than dietary supplements of crude sources of this factor Amounts as small as 6 micrograms per kilogram of diet had this effect. The optimal growth requirements appeared to be less than 30 micrograms per kilogram of diet.

539 LILLIE, R. J., DENTON C. A., and BIRD H. R. *Relation of vitamin B_{12} to the growth factor present in cow manure*, J Biol. Chem. 176 1477 1478, Dec. 1948 (Letter to Editor)

Crystalline vitamin B_{12} was found to have activity for chick growth equivalent to that of the unknown growth factor occurring in cow manure and in fish meal and some other feedstuffs of animal origin. The crystalline vitamin B_{12} was given orally or by injection into the breast muscle. The maximum growth response was the same for crystalline vitamin B_{12} and for the acid precipitate of water extract of cow manure and essentially the same for the crystalline vitamin and 2-unit liver extract. By calculation, it was found that the acid precipitate contained the equivalent of 5.8 mcg. of vitamin B_{12}. Comparative chick and bacterial assays would be of interest to determine the possible existence of different forms of the vitamin. The injection experiments are particularly significant, since they show that vitamin B_{12} has a direct effect on the chick, which is not mediated through the intestinal flora.

540. NICHOL, C. A., ROBBLEE, A. R., CRAVENS W. W., and ELVEHJEM, C. A. *The growth response of chicks to anti-pernicious anemia preparations*, J Biol. Chem. 177 631-639 Feb. 1949

A number of injectable liver extracts and crude liver fractions were tested for growth-stimulating potency in the chick. The stated U.S.P potency was not related to the growth response. Reticulogen (a liver extract) at 0.01 unit per bird per day caused a half maximal growth response. A vitamin B_{12} concentrate effectively stimulated chick growth at a level of 1.5 mcg. of vitamin B_{12} per 100 Gm. of ration. However the concentrate used did not exclude the presence of other factors such as those that occur in reticulogen. Other liver extracts (15 U.S.P units) active in the remission of pernicious anemia, were inactive for the growth of the chick. Two crude liver fractions which are discarded in the commercial preparation of anti-pernicious anemia extracts were very active in promoting chick growth.

541. OTT W H.: *Further studies of the activity of crystalline vitamin B_{12} for chick growth* (Paper presented at American Chemical Society meeting Symposium on vitamin B_{12} and related factors) Atlantic City Sept. 19-23 1949

Chicks from hens fed only an all-vegetable protein ration were given a diet consisting of 70 per cent soybean meal plus purified nutrients (vitamins, minerals, amino acids) known to be needed by the chick, and lacking only in nutrients having vitamin B_{12} activity Crystalline vitamin B_{12} was then added at various levels (the control group receiving none) and the growth responses determined. From the results obtained it was estimated that the minimum vitamin B_{12} requirement for maximum early growth of chicks was 2.7 mcg. per 100 Gm. of ration. A probable maintenance requirement of not less than 0.01 mcg. per 100 Gm. was indicated. The vitamin B_{12} requirements apparently were increased when dried whey alfalfa meal, and certain other natural products were included in the diet.

542. MILLER, D C., and GROSCHKE, A. C.: *The occurrence of "animal protein factor" in horse manure as measured by chick growth responses*, Michigan Agric. Exper Sta. Quart. Bull. 32 279-287 Feb. 1950.

Incubated horse manure was found to be a potent source of APF while fresh horse manure contained no activity (Same as previously reported for chick feces.) This shows that intestinal synthesis of APF does not occur in the horse. "This observation pointed out the importance of certain aerobic bacteria in the synthesis of APF after defecation and that synthesis of APF did not occur in the intestine of chickens."

A charcoal adsorbate of vitamin B_{12} was shown to have APF activity

543 COMBS G F., CARLSON C. W., MILLER, R. F., PEELER, H. T., NORRIS, L. C., and HEUSER, G. F: *Studies of unidentified chick growth factors*, J Biol. Chem. 182 727 737 Feb. 1950.

Studies of growth-stimulating factors in counter current distribution fractions obtained from refined liver paste dialysate indicate the existence of four unidentified substances which promote rapid early growth in chicks. An improved microbiologic assay with Lactobacillus leichmannii gave evidence that two of these factors are different forms of vitamin B_{12}. The other substances do not appear to be identical with vitamin B_{12} or with any of the known vitamins. Significant growth responses have been obtained by feeding fractions containing either of these unknown factors at levels which supplied approximately 0.03 to 0.04 mcg. of vitamin B_{12} per 100 Gm. of diet. This level of vitamin B_{12} is too low to have any material growth-promoting effect on chicks. A combination of fractions containing these two unknown factors appeared to indicate a mutual supplementary effect.

Lactobacillus leichmannii responds in the presence of adequate vitamin B_{12} to an unknown factor obtained from countercurrent distribution fractions of liver paste, which also contains one of the unknown chick growth factors. Lactobacillus casei also responds to these fractions. Whether or not the chick and microbiologic factors are identical has not been determined.

Cornell University
Ithaca, N Y

544 GASSNER, F X., PATTON A. R., WILGUS, H. S., and CHARKEY L. W *Failure of cockerel comb and testis development on sesame meal and its prevention by vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 75 630-633, Dec. 1950.

545. COUCH, J R., OLCESE, O., SANDERS B G., and HALICK, J V: *Vitamin B_{12}, APF concentrates dried whey fish solubles and liver fraction "L" in the nutrition of the mature fowl*, J Nutrition 42 473-485, Nov 1950.

546 MENGE, H., MORENG R. E., and COMBS, G F: *Effect of yolk removal in day-old chicks on early nutritional requirements*, Proc. Soc. Exper Biol. & Med. 76 46-49 Jan. 1951.

Rats

547 EMERSON G A., WURTZ, E., and ZANETTI, M. E.: *Vitamin B_{12}—a growth factor for young rats*, Federation Proc. 8 381-382, March 1949

Size and birth weights of the litters cast by rats that received 5 mcg. vitamin B_{12} daily from the time of impregnation were the same as those of litters cast by untreated rats. The weaning weights (at 8 days) of the offspring of the mothers receiving vitamin B_{12}, however averaged 50 per cent more than those of the offspring of the untreated mothers. At weaning, each group was separated into two groups, one of which received 0.5 mcg vitamin B_{12} daily for 90 days while the other received no supplement. The weight increments of the males from the different groups were as follows

Males from control mothers, young undosed	181 Gm.
Males from control mothers, young dosed	237 Gm.
Males from treated mothers, young undosed	279 Gm.
Males from treated mothers, young dosed	303 Gm.

The feeding of vitamin B_{12} to the young of control mothers resulted in body weights 100 Gm. in excess of those of the unsupplemented young of these mothers. Since the undosed young of the treated mothers grew for two months at a rate approximating that of the dosed young from the control mothers, it would appear that vitamin B_{12} is stored during the suckling period. The red and white cells of the individuals of all groups fell within the normal range.

548. HARTMAN A. M., DRYDEN L. P., and CARY C. A. *Bacterial synthesis in the rat of an unidentified growth-promoting factor* Federation Proc. 8 205, March 1949

Some rats on a diet deficient in an unidentified growth-promoting vitamin (X) supplemented with unusually high levels of the ten B vitamins plus methionine and vitamins C, E, and K, gained weight nearly twice as fast as others on the same diet. The slow-growing rats responded to a single small dose of feces from those that grew rapidly This suggests that X may be synthesized by microorganisms which may be induced to thrive in the rat. This synthesis was made possible by the extraordinarily high level of riboflavin. Findings reported by Stokstad, by Ott, and by Bird (Abstr 84 538, 539) are cited which suggest that the animal protein factor anti-pernicious anemia factor X, and vitamin B_{12} are either very closely related or identical. X occurs in different combinations in different feeds; all are active in the normal rat.

549 HARTMAN A. M., DRYDEN L. P., and CARY C. A. *A role of vitamin B_{12} in the normal mammal*, Arch. Biochem. 23 165-168, Aug. 1949

The authors have compared crystalline vitamin B_{12} with APA 15-unit liver extract fed to weanling young rats as supplements to X-deficient basal rations adequate in other nutrients and containing 25, 45, or 65 per cent protein. The test young were reared by mothers that were on a X-deficient ration during the nursing period. The 14-day growths on the basal ration containing an X deficient casein decreased from a weight gain of 36 Gm. to 22 Gm. and 4 Gm., respectively with these increases in the percentage of protein. Supplementation with vitamin B_{12} increased the rate of growth on all levels of protein, and maximally effective doses of vitamin B_{12} produced practically the same effect as doses of the liver extract which the authors previously had found effective. The results show that with all levels of protein tested a deficiency of vitamin B_{12} has a deleterious effect on growth. Apparently vitamin B_{12} plays a fundamental role affecting the capacity of the normal mammal to utilize protein.

550. PICCIONI, M., RABBI, A., and MORUZZI, G.: *Animal protein factor for the rat present in crude casein and its relationships with vitamin B_{12}*, Science 113 179-181, Feb. 16, 1951.

Crude casein contains a factor (or factors) which is not B_{12}, that is indispensable for the normal growth and reproduction of rats.

551 CUTHBERTSON W F J., and THORNTON D M. *Effect of parenteral nutrition on the growth of the rat to vitamin B_{12}*, Brit. M. J 5 xii, 1951.

Swine

552. HOGAN A. G., and ANDERSON G. C.: *Vitamin B_{12} in swine nutrition*, Federation Proc. 8 385-386, March 1949

Six pigs were taken from their mothers at the age of two days and reared on a synthetic milk diet including vitamin-free casein, sucrose, corn starch, lard, mineral salts, and generous supplies of vitamins. Three of them were given intramuscular injections of crystalline vitamin B_{12} at three-day intervals until they were 36 days old. The total dosage of B_{12} was 50 mcg. in one pig, 100 mcg. in the second, and 200 mcg. in the third. In the following four-day period the pigs that received vitamin B_{12} made an average gain of 26.8 pounds and the others gained 15.9 pounds. In the following six-week period one of the pigs that did not receive vitamin B_{12} died unexpectedly one did not gain consistently and the third began to decline but after injections of vitamin B_{12} totaling 15 mcg. began to gain at a moderate rate. The pigs that received vitamin B_{12} grew at a uniform rate, with an average gain of 58.8 pounds, which is exceptional for that age.

553. NEUMANN A. L., JAMES, M. F., KRIDER, J L., and JOHNSON, B. C.: *Essentiality of vitamin B_{12} for the baby pig, with preliminary quantitative data*, Federation Proc. 8 391 March 1949

The addition of a vitamin B_{12} concentrate to isolated soybean protein synthetic milk improved the growth and physiologic well-being of baby pigs. The maximum growth response was obtained with 42 mcg. per Kg. of dry matter in the diet, and hemopoiesis was improved by this diet. The vitamin B_{12}-deficient pigs were irritable, sensitive to touch, and sluggish in their movements; a number of them could not stand because of weakness in their rear legs. The pigs that received vitamin B_{12} at sufficient levels had none of these symptoms and grew more rapidly A manure factor concentrate gave good growth but did not protect against the gross symptoms observed in the vitamin B_{12}-deficient pigs.

554 JOHNSON B. C., and NEUMANN A. L. *Crystalline vitamin B_{12} compared to antipernicious anemia liver extract for pig growth*, J Biol. Chem. 178 1001 1002, April 1949

The responses of baby pigs to 2 mcg. of vitamin B_{12} and to reticulogen at 0.1 ml. per day were equal.

555 LUECKE, R. W., McMILLEN W N., THORP F., Jr., and BONIECE, J R. *The effect of vitamin B_{12} concentrate on the growth of weanling pigs fed corn-soybean diets*, Science 110 139-140 Aug. 5, 1949

Weanling pigs that were fed a corn and soybean diet supplemented with 0.5 per cent vitamin B_{12} concentrate gained weight at a significantly greater rate than pigs fed the same basal ration without the supplement. By the end of a seven week trial period they averaged 17 lbs. heavier than their controls. Only 2.65 lbs of supplemented feed was required to produce a 1 lb. gain in body weight compared with 2.94 lbs. of unsupplemented feed, which indicated that the concentrate effected more efficient utilization of the feed. It seemed probable that vitamin B_{12}

per se was responsible for the growth promoting activity of the concentrate, although it cannot be definitely stated that this was the case since the latter undoubtedly contained impurities.

556. ANDERSON G. C., and HOGAN A. G.: *Requirement of the pig for vitamin B_{12}.* J Nutrition 40 243-253, Feb. 1950.

The tentative estimate of the quantitative requirement of the pig for vitamin B_{12} when administered orally is 0.26 mcg. daily per Kg. of live weight, or not over 1.5 mcg per 100 Gm. of food.

Two pigs supplied with fortified cow's milk grew more rapidly than did those that received vitamin B_{12}. This suggests that an unrecognized vitamin is essential for the optimum nutritional state.

557 NEUMANN A. L., and JOHNSON B. C.: *Crystalline vitamin B_{12} in the nutrition of the baby pig* J Nutrition 40 403-414, March 1950

Crystalline vitamin B_{12} has been shown to be required by the baby pig. It is the growth factor which is supplied by the anti-pernicious anemia liver extract. The relation between the deficiency produced in baby pigs and pernicious anemia in man is discussed.

558. NESHEIM, R. O., KRIDER, J. L., and JOHNSON B. C. *The quantitative crystalline vitamin B_{12} requirement of the baby pig* Arch. Biochem. 27 240-242, June 1950.

Requirement of vitamin B_{12} given by injection (0.6 mcg./Kg. body weight) was found to be one half that by oral administration (20 mcg./Kg. of dry matter consumed)

559 BURNSIDE, J. E., CUNHA, T. J., EDWARDS H. M., MEADOWS G. B., LAMAR, G. A., PEARSON A. M., and GLASSCOCK, R. S. *Response of the pig to APF B_{12} and B_{12b}.* Proc. Soc. Exper Biol. & Med. 74 173-174, May 1950.

A mixture of B_{12} and B_{12b} was of no benefit for growth when administered by injection or orally to the pig during a 40-day period under the conditions of this experiment. APF prevented a diarrhea which occurred periodically with the other pigs on experiment. APF fed pigs exhibited a smoother hair coat and more bloom. Further evidence was obtained to show that APF contains a factor(s) other than B_{12} or B_{12b}.

560 BOOTH, A. N., ELVEHJEM, C. A., and HART E. B. *The importance of bulk in the nutrition of the guinea pig.* J Nutrition 37 263-274 Feb. 1949

A purified basal ration containing all the known nutrients, except vitamin B_{12}, produced a growth rate of only 1.8 Gm. per day for six weeks in guinea pigs compared to a normal rate of 6.9 Gm. per day when a commercial ration composed of natural feeds was fed. A nearly normal average growth rate of 6.5 Gm. per day was obtained when the purified basal ration was supplemented with 25 per cent alfalfa leaf meal in place of an equal amount of sucrose. Growth was equally good when the basal ration was supplemented with 30 per cent dried beet pulp. Powdered gum arabic fed at a level of 15 per cent produced an average growth rate of 5.1 Gm. per day. Nearly normal growth rates were obtained for periods as long as twelve weeks, on the 15 per cent gum arabic ration by the addition of the ash from alfalfa meal equivalent to 25 per cent. When gum arabic was fed, the level of protein in the synthetic diet could be reduced

University of Wisconsin Madison, Wis.

Other Animals

561 SCHEID H. E., McBRIDE, B. H., and SCHWEIGERT B. S.: *The vitamin B_{12} requirement of the Syrian hamster* Proc. Soc. Exper Biol. & Med. 75 236-239 Oct. 1950

Syrian hamsters were kept on either a corn-soybean oil meal ration with or without iodinated casein, or a casein basal ration with or without iodinated casein for a two-week depletion period. Each group was then divided into three subgroups, which received either the basal ration, the basal ration plus 50 mcg. of vitamin B_{12} per Kg. of ration, or the basal ration plus 2 per cent whole liver substance (which may provide additional unknown factors as well as vitamin B_{12}) These supplements produced a small gain in weight only in the animals on the corn-soybean and iodinated casein diet in the other groups there was either no difference in growth or slightly less growth in the animals receiving the supplements. A vitamin B_{12} requirement of the hamster therefore, was not demonstrated.

In parallel experiments with rats on the corn-soybean oil meal ration plus iodinated casein, a vitamin B_{12} requirement was demonstrated. A reduction in food efficiency attributable to vitamin B_{12} deficiency was observed in these animals.

American Meat Institute Foundation, and The University of Chicago Chicago, Ill.

562. SCHWEIGERT B. S.: *The animal protein factor* Nutrition Rev 7 225-227 Aug. 1949

The author provides a list of the animal protein factors that have been studied most extensively including their sources, and discusses their occurrence, the nature of the nutritional deficiencies produced in various animals, and interpretations of the results obtained. Ample evidence is available demonstrating that unknown factors are required by the chick; vitamin B_{12} is capable of replacing the active fractions from cow manure, liver extract, and fish solubles, either with a soybean oil meal-corn or casein basal ration. It is pointed out that the various animal protein factors may be attributable to one chemical entity or its conjugates, as in the case of folic acid. The factors replaceable by folic acid are tabulated. It is suggested that a similar tabulation for factors with activity attributable to vitamin B_{12} may be made in the near future.

563 ZUCKER, T. F., and ZUCKER, L. M. *"Animal protein factor" and vitamin B_{12} in the nutrition of animals* Vitamins & Hormones 8 1 54, 195

564 ASCHKENASY A.: *La vitamine B_{12} et le facteur des protéines animales (Vitamin B_{12} and the animal protein factor)* Ann. Nutrit. Aliment. 4 141 167 1950

COBALT REQUIREMENT OF SHEEP

565. BECKER, D E., SMITH, S. E., and LOOSLI, J K. *Vitamin B_{12} and cobalt deficiency in sheep*, Science 110 71 72, July 15, 1949

Cobalt-deficient lambs had been found to respond quickly by improved appetite, weight gain, and increased blood hemoglobin when fed, but not when injected, with 1 mg. of cobalt daily This suggested, assuming vitamin B_{12} to be a necessary metabolite for sheep, that cobalt was synthesized into vitamin B_{12} by the rumen flora when it was administered orally but that injected cobalt was incapable of this transformation. A study was therefore made of the effect of oral or intramuscularly injected vitamin B_{12} in cobalt-deficient lambs. The dosage of the material was arbitrary one animal receiving as little as 2 micrograms and another as much as 120 micrograms weekly The larger amounts were administered orally Injections were given twice weekly Regardless of the amount of the dosage, neither route of administration produced a response over periods of five to six weeks, as judged by hemoglobin levels and weight gains. The lack of response to vitamin B_{12} in these tests lends no support to the theory that this factor is an important intermediary in cobalt metabolism in lambs.

566. MARSTON H. R., and LEE, H. J : *Primary site of the action of cobalt in ruminants* Nature 164: 529-530, Sept. 24, 1949 (Letter to Editor)

More than 10 years ago the authors discovered that cobalt, given orally was dramatically effective in coast disease, a fatal malady affecting sheep confined to cobalt deficient pastures. Their later experiments have shown unequivocally that for the cobalt to be effective it must be ingested, and that cobalt introduced parenterally is of no benefit. They conclude that cobalt exerts its influence on the ruminant primarily either in the lumen of the alimentary canal or when passing through its wall, the site of its activity being apparently above the level of the duodenum since cobalt given intravenously appears in the intestinal tract below this level. A probable important function of cobalt in the ruminant may concern the activity of the symbiotic flora within the paunch. Sheep and cattle are apparently the only animals affected by cobalt-deficient grazing land rabbits thrive on it and horses do exceptionally well. This implies that the ruminant is unique in its higher demand for the element.

The authors refer to evidence that neither the anemia nor other symptoms of extreme cobalt deficiency respond to Minot s anti-pernicious anemia factor introduced parenterally either as concentrated liver extract or as crystalline vitamin B_{12}, in quantities that would rapidly correct the dyscrasia of human pernicious anemia. It would now appear that the physiologic role of cobalt is not restricted to the part it takes in the maturation of erythrocytes in the bone marrow

Council for Scientific and Industrial Research Adelaide, Australia

567 ABELSON P H., and DARBY H. H. *The synthesis of vitamin B_{12} in the digestive system of the sheep*, Science 110 566, Nov 25, 1949

About one half the amount of cobalt fed to sheep was excreted in organically bound form in the feces.

Biological assay of the butanol extract indicated the presence of large amounts of vitamin B_{12} thus sheep feces appear to be an important source of this vitamin.

Carnegie Institution of Washington Washington, D C.

568. HARPER, A. E., RICHARD R. M., and COLLINS, R. A. *The influence of dietary cobalt upon the vitamin B_{12} content of ewe s milk*, Arch. Biochem. & Biophysics 31 328-329 April 1951.

The supplementation of cobalt or trace-minerals (containing cobalt) to the ration of ewes produced a highly significant increase in the vitamin B_{12} content of the ewe s milk. It is probable that this observation is the result of increased vitamin B_{12} synthesis by the rumen microorganisms.

569 SMITH, S. E., KOCH, B. A., and TURK, K. L.: *The response of cobalt-deficient lambs to liver extract and vitamin B_{12}*. J Nutrition 44: 455-464, July 1951.

The curative activity of an anti-pernicious anemia liver extract when injected into cobalt-deficient lambs has been confirmed. There was a high correlation between the vitamin B_{12} activity of these liver extract fractions and the response of the deficient animals. The presently available data show that vitamin B_{12} is an important intermediary in cobalt metabolism in sheep.

FOLIC ACID; OTHER VITAMINS

570 NICHOL, C. A., DIETRICH, L. S., ELVEHJEM, C. A., and HART E. B.: *Observations on folic acid deficiency in the chick in the presence of vitamin B_{12}*. J Nutrition 39 287 298, Nov 1949

Folic acid deficiency in chicks was accentuated by oral or parenteral administration of vitamin B_{12}. Folic acid treatment completely counteracted the symptoms within 24 hours. B_{12} increased the growth rate but not the feathering in folic acid-deficient chicks.

571. DIETRICH, L. S., NICHOL, C. A., MONSON, W J., and ELVEHJEM, C. A.: *Observations on the interrelationship of vitamin B_{12}, folic acid and vitamin C in the chick*, J Biol. Chem. 181: 915-920, Dec. 1949

Vitamin C and B_{12} stimulate growth of chicks fed a folic acid-deficient diet.

Vitamins C and B_{12} stimulate synthesis of folic acid folic acid stimulates the synthesis of B_{12}.

572. EVANS R. J., GROSCHKE, A. C., and BUTTS, H. A. *Effect of vitamin B_{12} on pantothenic acid metabolism in the chick*, Arch. Biochem. & Biophysics 31 454-456, May 1951.

Chicks that were more deficient in vitamin B_{12} had higher liver pantothenic acid contents.

"Our tentative explanation of the data obtained is that vitamin B_{12} aids in the transfer of pantothenic acid from the liver for use elsewhere in the body and that in the absence of sufficient vitamin B_{12}, pantothenic acid accumulates in the liver"

573. YACOWITZ, H., NORRIS, L. C., and HEUSER, G F: *Evidence for an interrelationship between vitamin B_{12} and pantothenic acid* J Biol. Chem. 192 141 146, Sept. 1951

Vitamin B_{12} was found to spare the pantothenic acid requirement for growth, survival, and the prevention of dermatosis in chicks with normal reserves of vitamin B_{12}.

In turn, pantothenic acid showed a sparing effect on the vitamin B_{12} requirement for growth of normal chicks.

References are given for interrelationships between vitamin B_{12} and amino acids in general methionine, glycine, choline, betaine, folic acid and ascorbic acid.

Most pantothenic acid in tissues is present in coenzyme A.

574 HOVE, E. L., and HARDIN J O *Relation of vitamin B_{12} to vitamin E in nutrition of young rats* Federation Proc. 9 362, March 1950.

"Previous work has shown that supplements of alpha tocopherol given to young rats on a low casein, vitamin E-free diet increased growth and protein utilization, gave marked protection against CCl_4 and prevented a fatal lung hemorrhage–liver necrosis syndrome which occurred after about 3 months. The diets used in this work contained no added source of vitamin B_{12}, folic acid or biotin. (Hove, E. L., D H. Copeland and W D Salmon. *J Nutrition* 39: 397 1949) When boiled egg white or fibrin replaced casein as the protein source at 10% supplements of 1 mg. alpha tocopherol daily did not increase growth or give protection against CCl_4. However on diets with defatted whole wheat or soybean meal as the protein source, vitamin E increased growth and protein utilization. When vitamin B_{12} concentrate was added at a level equivalent to 50 µg /kg. to diets containing soybean meal, casein or whole wheat, the protein efficiency ratios increased sharply and vitamin E supplements were without apparent benefit. Folic acid or biotin did not influence the results. Supplements of vitamin B_{12} offered some protection against CCl_4, but under the conditions used could not replace vitamin E the combination was more effective than either alone. Against the fatal lung hemorrhage–liver necrosis syndrome vitamin B_{12} gave little protection although in some experiments the time of onset was delayed."

Alabama Polytechnic Institute Auburn, Ala.

575 DINNING J S., KEITH, C. K., PARSONS J T., and DAY P L. *The influence of pteroylglutamic acid and vitamin B_{12} on the metabolism of pyridine-fed rats* J Nutrition 42 81-88, Sept. 1950.

576. ERSHOFF B. H., and McWILLIAMS H. B.: *Effects of B vitamins and liver on growth of immature rats maintained at low temperatures* Proc. Soc. Exper Biol. & Med. 75 226-229 Oct. 1950.

Immature rats kept in low temperatures were fed a purified ration supplemented, in some instances, with desiccated whole liver, water insoluble liver residue, or a water-soluble liver extract. Body weight and gonadal weight increased less rapidly in the control rats kept in the cold room than in those under room temperature conditions. The supplements resulted in a marked increase in both body and gonadal weight. A supplement of the known B vitamins was similarly effective. It is suggested that the protective effects of the various supplements were due, at least in part, to their vitamin B_{12} content.

Emory W. Thurston Laboratories Los Angeles, Calif.

577 VERLY W G., WILSON J E., KINNEY J M., and RACHELE, J R. *Action of vitamin B_{12} and folic acid on labile methyl synthesis in the rat,* Federation Proc. 10 264 March 1951.

"It has been shown that the carbon of methanol can be incorporated into the methyl group of choline in the rat (du Vigneaud and Verly *J Am. Chem. Soc.* 72 1049 1950) Experiments with methanol labeled with deuterium and C^{14} indicated that methanol was used, presumably through oxidation and subsequent reduction, in the synthesis of a methyl group in the formation of a labile methyl compound (du Vigneaud, Verly Wilson, Rachele, Ressler and Kinney *J Am. Chem. Soc.* In press) This newly formed methyl group then enters transmethylation reactions. Experiments have now been undertaken to see whether vitamin B_{12} or folic acid had any effect on the utilization of methanol. C^{14} Deuteriomethanol was injected into rats subcutaneously Trimethylamine derived from choline isolated from the carcasses was analyzed for C^{14} and deuterium. No significant difference in the utilization of methanol for labile methyl synthesis was found between young, B_{12}-deficient rats and their litter mates prepared in the same way but injected with B_{12} prior to administration of methanol. Folic acid administration caused a two-to-threefold increase in the utilization of methanol in labile methyl synthesis in the folic acid-deficient rat. These results on B_{12} and folic acid supplement those obtained by Sakami and Welch (*J Biol. Chem.* 187: 379 1950) on labile methyl synthesis from formate in liver slices."

Cornell University Medical College New York, N Y

578. HARTMAN, A. M., DRYDEN L. P., and CARY C. A.: *The effect of riboflavin on the bacterial synthesis of vitamin B_{12}-active material in the rat,* Arch. Biochem. & Biophysics 34 324-338, Dec. 1951.

The results of this study indicated bacterial synthesis of B_{12}-active material in the intestinal tract which was shown to be due to high levels of dietary riboflavin.

579 ENGEL, R. W., and ALEXANDER, H. D: *Prevention of nutritional edema with vitamin B_{12} and folic acid,* Federation Proc. 11 441-442, March 195

"A choline-low diet containing 7% of protein produced a high incidence of generalized nutritional edema and severe anemia in weanling rats in an average period of 90 days. The edema and anemia were prevented by supplementing the diet with 0.2% of choline chloride (Engel and Alexander *Federation Proc.* 10 382, 1951) The edema was completely prevented and hemoglobin levels of 11 gm./100 ml. of blood were maintained for 300 days when the choline-low diet was supplemented with 30 µg. of vitamin B_{12} and 2 mg. of folic acid/kg. of diet. Rats receiving this supplement made average body weight gains of 120 gm. during the experiment, contrasted with average body weight gains of only 20 gm. in rats protected from edema with a supplement of choline. A supplement of folic acid alone did not prevent the edema or the anemia although it appeared to prolong survival. Rats receiving this supplement failed to grow and developed edema in 120 days, at which time the blood hemoglobin levels had decreased to between 2.2 and 6.1 gm/100 ml. of blood."

Alabama Polytechnic Institute
Auburn, Ala.

580. TOVE, S B., and ELVEHJEM, C. A. *Relation of factor in a methanol extract of liver and pteroylglutamic acid in mink*, Federation Proc. 8 399 March 1949

In the experiments described, it was found that the pteroylglutamic acid deficiency syndrome in mink can be relieved or prevented by administration of a methanol extract of fresh liver The pteroylglutamic acid content of blood, urine and feces of the animals was greater than could be accounted for by the pteroylglutamic acid in the extract. Vitamin B_{12} preparations or commercial liver extracts used in pernicious anemia did not produce the response obtained with the methanol extract. This indicates that the methanol extract factor which is different from vitamin B_{12}, has a sparing action on pteroylglutamic acid.

581 TOVE, S. B., LALOR, R. J., and ELVEHJEM, C. A.: *Properties of the methanol soluble factor required by the mink*, Proc. Soc. Exper Biol. & Med. 75 71 74, Oct. 1950

The methanol extract factor required by the mink is soluble in 90 per cent phenol and insoluble in acetone, n-butanol and ¾ saturated ammonium sulfate. It is contained in fish solubles, but not in dried distillers solubles, dried whey pork spleen, or hog intestinal mucosa. It will be noted that there is a good correlation between the content of this factor and of vitamin B_{12} in the various products. The solubilities of this factor and of vitamin B_{12} are similar The classic symptoms of pernicious anemia are seen in mink deficient in the methanol extract factor In the chick and the rat, vitamin B_{12} has been shown to be effective in preventing fatty degeneration of the liver and kidneys a similar condition in the mink is cured by the methanol extract. Since the methanol extract is active at a daily intake of less than 1 cc., which contains only 2 mcg. of crystalline vitamin B_{12}, while the crystalline vitamin B_{12} is effective only in doses of 10 to 20 mcg., the methanol extract factor must have an effect in addition to the actual amount of B_{12} present. It would seem that the methanol extract factor may be another form of vitamin B_{12}.

University of Wisconsin
Madison, Wis.

582. JOHNSON B. C., NEUMANN A. L., NESHEIM, R. O., JAMES M. F., KRIDER, J L., DANA, A. S., and THIERSCH, J B.: *The interrelationship of vitamin B_{12} and folic acid in the baby pig* J Lab. & Clin. Med. 36 537-546 Oct. 1950.

Authors summary "Baby pigs fed a vitamin B_{12}-deficient diet for three weeks were co-fed 8 Gm. of x methyl folic acid per kilogram of dry matter of the diet for the next two weeks, which resulted in a marked decrease in growth rate and in the death of five out of twelve pigs.

"At the end of the three-week depletion period, the bone marrow showed a rise in nucleated red cells. Following the two-week feeding of the folic acid antagonist the bone marrow became depleted, especially in erythroid elements, and numerous basophilic normoblasts and erythroblasts appeared.

"The blood and bone marrow symptoms of this combined vitamin B_{12}-folic acid deficiency were cured by either crystalline vitamin B_{12} or by folic acid therapy

"Vitamin B_{12} therapy resulted in optimum growth, while folic acid treatment gave only temporary and suboptimal growth stimulation.

"In a second experiment baby pigs were fed a lower protein (20 per cent as compared with 30 per cent in the previous experiment) vitamin B_{12}-folic acid low diet plus Sulfathalidine in an attempt to produce the double deficiency without the use of a folic acid antagonist.

"In this experiment a marked reticulocyte response occurred on vitamin B_{12} therapy followed by a second marked response to folic acid administration.

"The vitamin B_{12}-deficient pigs were found to have enlarged thyroids, kidneys, livers, and tongues.

"From these two experiments it appears that the pig requires both vitamin B_{12} and folic acid and that both are involved in hematopoiesis. In addition, vitamin B_{12} is required for normal growth."

University of Illinois
Urbana, Ill.
Sloan-Kettering Institute for Cancer Research
New York, N Y

583 REID M. E. *Development of a synthetic diet for young guinea pigs and its use in the production of specific B-vitamin deficiencies* Federation Proc. 12 473, March 1953.

"A synthetic diet containing vitamin-free casein, sucrose, corn starch, cerelose, cellophane, corn oil, minerals and vitamins has been developed for guinea pigs, starting them at 2-4 days of age and continuing up to 8 weeks of age. With respect to general health, rate of growth, appearance and size of the internal organs, and the blood picture, this diet produced animals similar to *a*) those reared on the same diet supplemented with kale, *b*) those on a commercial rabbit chow diet with ascorbic acid

added, and c) those on *diet b* but supplemented with kale. The omission of individual B vitamins from the diet in different experiments, started with guinea pigs 2-4 days old, has led to the production of clear-cut deficiencies of thiamine, niacin, choline, pantothenic acid pyridoxine, folic acid, and riboflavin. Present results suggest that vitamin B_{12} and inositol are not essential for survival but may be essential for maximum growth. It appears that biotin in the diet may not be needed but additional studies are necessary The quantitative requirement of the different B vitamins is being determined."

National Institutes of Health
Bethesda, Md.

584 FATTERPAKER, P., MARFATIA, U., and SREENIVASAN A.: *Influence of folic acid and vitamin B_{12} on formation of creatine in vitro and in vivo* Nature 167 1067 1068, June 30, 1951

In Swiss mice, deficiency of either folic acid or vitamin B_{12} causes a diminution in creatine formation and excretion together their effects are additive. There is reason to believe that creatine formation is primarily influenced by folic acid and that vitamin B_{12} exerts its effect by promoting better utilization of folic acid at low levels. It is possible that in the absence of folic acid there is a drain on the methyl economy due to methionine and choline, giving rise to formate that formate utilization is favored by folic acid is now known. The favorable effect of vitamin B_{12} could not be observed in attempts at in vitro activation of the creatine-forming system by additions of vitamin B_{12} solutions.

University of Bombay
Matunga, Bombay 19 India

585 OLESON J J: *Studies on pteroylglutamic acid inhibitors* Trans. N Y Acad. Sc. 12 118-121, Feb. 1950.

Biological studies on a number of PGA derivatives are reported. Certain interrelationships between PGA derivatives and dietary protein and vitamin B_{12} have been illustrated.

PROTEINS, FATS CARBOHYDRATES

586. HARTMAN A. M., DRYDEN L. P., and CARY C. A. *The role and sources of vitamin B_{12}*, J Am. Dietet. A. 25 929-933, Nov 1949.

The authors fed various diets deficient in vitamin B_{12} to weanling rats. It was found that incredibly small doses of B_{12} (as low as 0.01 mcg. daily) suffice to produce significant increases in the rate of growth of rats. The feeding of B_{12}-deficient rations with high levels of protein was deleterious—sometimes even fatal. It is, therefore, concluded that vitamin B_{12} plays an important role in determining the capacity of the normal mammal to utilize protein. Furthermore, vitamin B_{12} plays an important role in the growth and development—and probably in the reproduction and lactation—of the normal mammal. Various foods and feeds tested for B_{12} activity by feeding them in, or as a supplement to, basal rations are shown in the accompanying table.

FOODS AND FEEDS

Without B_{12} ACTIVITY	*With* B_{12} ACTIVITY
White flour	Milk
Enriched white flour	Skim milk (liquid or dried)
Whole wheat flour	Cheese (cottage, Swiss, Cheddar)
Wheat bran	Casein, crude
Yeast (bakers' or brewers')	Liver extracts
Cornmeal (yellow)	Beef muscle
Soybean oil meal	Pork muscle
Linseed oil meal	Egg yolk
Corn silage	Lettuce
Distillers' dried solubles	Alfalfa or alfalfa hay
Cottonseed flour (deglanded, defatted)	Timothy hay
Carrots	Kentucky blue grass
Tomatoes	Rice polishings concentrate
Egg white (heat-coagulated)	
Butterfat	

The authors have calculated that 1 quart of milk, when fed to a normal rat, has a potency equivalent of 10 or 12 mcg. of crystalline B_{12}.

U S Department of Agriculture
Beltsville, Md.

587 CUNHA, T J., BURNSIDE, J E., EDWARDS, H. M., MEADOWS, G B., BENSON R. H., PEARSON A. M., and GLASSCOCK, R. S *Effect of animal protein factor on lowering protein needs of the pig*, Arch. Biochem. 25 455-457 Feb. 1950.

Efficient utilization of protein by pigs was obtained only when the diet was supplemented by APF

The accepted values for the protein requirements of swine may need to be reevaluated by using adequate amounts of vitamin B_{12}, plus other factors present in the APF supplement in the ration.

588. KRZYWICKI H., and DACOSTA, E. *Effect of high protein unsupplemented and the same supplemented by B_{12} after dietary restriction on the spontaneous activity of the rat*, Am. J Physiol. 171 741, Dec. 1952 (in Soc. Proc.)

"Spontaneous activity body weight and food intake were studied on 96 rats during either *diet 1* (caloric restriction) *diet 2* (2% protein) *diet 3* (4.5% protein and 10% salt) and during rehabilitation on *diet 4* (60% protein ad libitum) *diet 5 (diet 4* limited to 11 gms/day) and *diet 6 (diet 4* plus B_{12}) During rehabilitation after *diet 1* the activity of the group on *diet 5* rose during the first 9 weeks to 2355 revolutions but at the end of 13 weeks all groups were at about the same level (627 revolutions) The activity of the groups on *diet 6* and *4* after *diet 2* rose rapidly together for the first 10 weeks. After 13 weeks of rehabilitation the activity of the groups on *diet 6* was 3373 revolutions compared to 4244 for the *diet 4* group and 1371 for the *diet 5* group. During rehabilitation after *diet 3* the activity of the group on *diet 5* was greater than that of the other rehabilitants but all were below the controls. Thus, the previous dietary history determines the activity response during rehabilitation on a high protein diet."

U S Army Medical Nutrition Laboratory
Chicago, Ill.

589 HENRY K. M., and KON S. K.: *Vitamin B_{12} and the biological value of proteins*, Biochem. J 48 xi, Jan. 1951 (in Proc. Biochem. Soc.)

590. MARFATIA, U., and SREENIVASAN A. *Influence of vitamin B_{12} on the biological value of low quality protein diet*, Current Sc., Bangalore 20 128-129 May 1951

591. CHOW B. F., and BARROWS L. *Role of B_{12} on nitrogen retention of rats fed on soy bean protein diets at different caloric levels*, Federation Proc. 9 354 March 1950.

"Supplementation of vitamin B_{12} to soy bean protein diet accelerates the rate of growth of young rats deficient of this vitamin. It is therefore of interest to ascertain whether B_{12} enhances the efficiency of utilization of soy proteins. To this end, nitrogen balance experiments were performed to determine the amount of nitrogen retained by 2 groups of B_{12}-deficient rats, with and without supplementation of B_{12}. Their basal diet consisted of 40% sucrose and 60% Sobee, a commercial product containing 32% soy protein. Adequate vitamin supplement was also given. Urine and stool samples were collected weekly and analyzed for nitrogen, for a period of 6-8 weeks. It was found that when both groups of animals were given a restricted intake (6-8 gm. of diet/rat/day) supplementation of vitamin B_{12} did not increase the growth rate nor the retention of nitrogen. When the dietary allowance was increased by 50% vitamin B_{12} brought about a greater rate of growth but no better protein utilization. This is interesting since the added growth points to the effect of B_{12} on carbohydrate or fat metabolism rather than protein metabolism. In another experiment the daily ration was further increased to ad lib feeding. The growth rate but not the efficiency of nitrogen utilization was increased by the addition of this vitamin. These results therefore indicate that B_{12} does not enhance the biological value of soy bean proteins under our experimental conditions but may play an important role in carbohydrate or fat metabolism."

Johns Hopkins University
Baltimore, Md.

592. DACOSTA, E., and CLAYTON R.: *Serum cholinesterase of male albino rat during food restriction and subsequent protein rehabilitation*, Federation Proc. 10 379 March 1951

"Serum cholinesterase activity was measured in 182 male albino rats after 5, 10 or 20 weeks on a control, high protein (60%) low calorie or modified carrot (2% protein) diet by a decrease in pH per hour (ΔpH/hr) The average ΔpH/hr for rats on the control, high protein, low calorie or the modified carrot diet was 0.235, 0.259 0.254 and 0.191, respectively After 5 and 10 weeks on either the low calorie or modified carrot diet rats were rehabilitated on either a high protein diet *ad libitum* or with B_{12} (2.5 µg/rat) or 11 gm/rat/day Serum cholinesterase was determined after ¾ 2, 3, 7, 35 and 70 days of rehabilitation. After 18 hours of rehabilitation with the high protein diet *ad libitum* following 5 weeks on the low calorie diet a ΔpH/hr of 0.375 was found and after 10 weeks, 0.325. After 1 week of rehabilitation with a high protein diet *ad libitum* and with B_{12} following 10 weeks on the modified carrot diet an average ΔpH/hr of 0.328 and 0.334 respectively was found, otherwise the trend was toward the control level. Since changes in serum cholinesterase activity and body weight usually parallel each other this test may serve as an estimate of the nutritive state of an animal."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

593. DACOSTA, E., and CLAYTON R.: *Serum alkaline phosphatase of male rats during food restriction and subsequent protein rehabilitation*, Federation Proc. 11 439-440, March 1952.

"Serum alkaline phosphatase activity measured in 288 male rats after 35 or 70 days on a control, high (60%) protein, low calorie, high (10%) salt, or modified carrot (2% protein) diet was 65.6, 82.9 56.6, 114.6 and 25.3 units/100 ml. of serum, respectively After either 35 or 70 days of restriction, the rats were rehabilitated for ¾ 2, 3, 7 35 or 70 days on either a high protein diet fed ad libitum, or supplemented by B_{12} (7.5 µg/rat) or 11 gm/rat/day Serum alkaline phosphatase activity rose gradually or fell during early rehabilitation. After 7 days of rehabilitation following 35 days on the low calorie diet, serum alkaline phosphatase rose from 68 to 85% above the low calorie level and after 7 days of rehabilitation following 35 days on the carrot diet, serum alkaline phosphatase rose from 68-143% After 7 days rehabilitation following 35 days on the high salt diet, alkaline phosphatase was below the high salt level, but after 70 days of rehabilitation, from 30-41% above. The slowest recovery of serum alkaline phosphatase occurred in the groups given B_{12}. After 35 days of rehabilitation, it reached the control level in most rats. After 70 days, it mostly exceeded the high protein control level. A parallel rise in body weight and muscle and heart protein suggests that serum alkaline phosphatase activity serves as a measure of nutritional status."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

594 CLAYTON R., and DACOSTA, E.: *Liver glycogen of the rat after food restriction and rehabilitation on a high protein diet unsupplemented or supplemented by vitamin B_{12}*, Federation Proc. 11 438, March 1952.

"The average liver glycogen of 288 male rats fed either a control high (60%) protein, low calorie high (10%) salt, or modified carrot (2% protein) diet for 35-70 days was 2.5, 2.0, 1.2, 2.0 and 4.3 gm/100 gm. of tissue, respectively Restricted animals were rehabilitated for ¾ 2, 3, 7 35 or 70 days. After ¾ day of rehabilitation following 35 days on the low calorie diet, the liver glycogen rose to 140 to 440% above the low calorie level. After 7 days of rehabilitation following 70 days on the low calorie diet, liver glycogen varied from 140% above to 50% below the low calorie level. After 35 days on the carrot diet, the liver glycogen with ¾ 7 days of rehabilitation, was reduced to 5-22% of the depletion level. After 35 or 70 days of rehabilitation following 35 or 70 days on a low calorie diet, liver glycogen was essentially at the control level. After protein depletion and subsequent rehabilitation during a similar period, liver glycogen was significantly above the control level. In early rehabilitation after caloric restriction, increased gluconeogenesis may result from increased adrenocortical activity since liver glycogen gains and increased adrenal weight paralleled each other Increased glycogen deposition occurred later in rehabilitation following protein depletion, probably because time was needed to build up protein reserves for hormone synthesis."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

595. DaCOSTA, E., and CLAYTON, R.: *Total protein and fat of male rat carcasses after food restriction and subsequent protein rehabilitation,* Am. J Physiol. 167 777 Dec. 1951 (in Soc. Proc.)

"The average total protein and fat of the carcasses of 288 rats fed either a control, high protein (60%) or 3 types of restricted diets for 5 or 10 weeks were determined. Restricted animals were rehabilitated for ¾, 2, 3, 7 35 and 70 days on either a high protein diet *ad libitum,* 11 gm/rat/day or plus B_{12}. After 5 weeks on 1) low calorie, 2) carrot or 3) high salt diets, total carcass protein of rats rehabilitated for 2 days on high protein *ad libitum* was 19.5, 23.4 and 17.1 gm. % and the fat, 9.0, 10.0, 12.6 gm.% respectively After 2 days on high protein, 11 gm/rat/day total carcass protein was 26.7 28.1 and 20.5 gm.% and the fat 4.8, 8.7 and 10.0 gm.%, respectively After 2 days on high protein plus B_{12}, the total carcass protein was 24.8, 24.7 and 18.8 gm.% and the fat 4.0, 9 4 and 8.1 gm.% respectively After 10 weeks restriction and the above pattern of rehabilitation including 10 weeks, total carcass protein and fat of rats rehabilitated on high protein plus B_{12}, or 11 gm/rat/day showed the same relatively high protein and low fat. These findings suggest that a limited protein intake or unlimited protein plus B_{12} results in increased protein storage and decreased fat deposition."

U. S. Army Medical Nutrition Laboratory Chicago, Ill.

596. BOSSHARDT D K., PAUL, W J., and BARNES, R. H. *The influence of diet composition on vitamin B_{12} activity in mice,* J Nutrition 40 595-604, April 1950.

A decrease in the fat level of the diet intensifies the growth retardation due to a deficiency of vitamin B_{12}. This growth retardation may be partially corrected by the feeding of fat or by the administration of vitamin B_{12}. These effects are intensified by increases in the protein level of the diet.

597 McCOLLUM, E. B., and CHOW B. F *Sex differences in weight stimulating effect of B_{12} in rats on diets of varying composition,* Proc. Soc. Exper Biol. & Med. 75 20-23, Oct. 1950.

Young growing rats which were the offspring of adults on a B_{12} deficient diet and therefore had reduced stores of this vitamin, were divided into four groups and given four diets varying in fat and carbohydrate content. Half of the animals in each group were given 0.5 mcg. of vitamin B_{12} subcutaneously three times a week. Comparison of the average weight gains showed that on all four of the rations studied the animals that received vitamin B_{12} gained more weight than the others. This was true of both sexes. When the calories were derived primarily from carbohydrate, vitamin B_{12} exerted a greater effect on weight in the females than in their littermate males. The weight-stimulating effect of B_{12} was greater in the females on high carbohydrate diets than in littermate sisters on diets of like protein content but with the calories provided by fat or a mixture of fat and carbohydrate.

Johns Hopkins University Baltimore, Md.

598. MONSON W J., DIETRICH, L. S., and ELVEHJEM, C. A.: *Studies on the effect of different carbohydrates on chick growth* Proc. Soc. Exper Biol. & Med. 75 256-259 Oct. 1950.

Chicks fed basal rations containing different carbohydrates grew most rapidly on dextrin, next on cerelose, next on sucrose, and least on lactose. Omission of folic acid from the basal rations resulted in a decrease in growth in all cases. This decrease was least on the lactose diet, but the birds receiving dextrin still showed the best growth. This confirms the findings of Luckey *et al.* that the chick requires less folic acid on rations containing dextrin than on rations containing sucrose.

Daily injections with a low level of vitamin B_{12} (0.1 mcg.) gave slight responses with all carbohydrates tested higher levels (0.6 and 1.0 mcg.) gave greater responses with lactose than with the other carbohydrates. The lactose-fed birds also give the greatest response to reticulogen and fish solubles. Neither these substances nor cellulose and sulfasuxidine changed the significant differences observed when the different carbohydrates are fed. Dextrin was retained longest in the body then sucrose, and lactose. The possibility of these nutritional differences being explained by the synthesis of known and/or unknown factors is discussed.

University of Wisconsin Madison, Wis.

599 LING, C. T., and CHOW B. F: *Observations on some biochemical changes induced by administration of vitamin B_{12} to deficient animals,* Federation Proc. 10 216, March 1951

"Although our knowledge of the metabolic role of vitamin B_{12} is meager experimental results point to its possible relationship to carbohydrate or fat metabolism. In order to throw some light on this subject, experiments were performed so as to follow some biochemical changes induced as a result of administering vitamin B_{12} to deficient animals. Rats from parents raised on soybean diet were used in all experiments. Each series of experiments consisted of a deficient and a B_{12}-injected group. Vitamin B_{12} was given subcutaneously to the latter in dosages ranging from 25 milligamma/day to 0.5 gamma 3 x a week for as long as 12 weeks. During the period of injection, blood sugar levels after varying period of fasting, were determined on both groups of animals. Blood sulfhydryl content was measured by the amperometric titration method. At the end of the experimental period, the animals were bled by cardial puncture and sacrificed. Glycogen, fat, and nitrogen content of the liver and total nitrogen, fat, and muscle glycogen content of the carcasses were analyzed. The results show that the deficient animals had much lower body fat, and higher water content, and lower blood sulfhydryl content, as compared to their litter mates on the same diet but receiving B_{12}-injection The significance of these findings is discussed in the light of the role of vitamin B_{12} in carbohydrate, fat, and protein metabolism."

Johns Hopkins University Baltimore, Md.

600. LING, C. T., and CHOW B. F: *Effect of vitamin B_{12} on the body composition of rats,* J Biol. Chem. 198 439- Sept. 1952 (abstr J Am. Dietet. A. 29 58, Jan. 1953.)

"It has been suggested that vitamin B_{12} may be involved in the utilization of carbohydrates and their transformation to fat rather than in protein metabolism. To study the theory further weanling young of vitamin B_{12}-deficient female rats were maintained on diets deficient in the vitamin, some being given crystalline vitamin B_{12} subcutaneously Administration of the vitamin enhanced the growth rate and increased the food intake. The bodies of animals with vitamin B_{12} deficiency were shown to have low fat, high water and normal protein contents. Upon chemical analyses, livers of the control and injected groups showed no significant differences in percentage composition of glycogen, nitrogen, or fat. However in the deficient animals the body weight was smaller relative to the liver weight in comparison with the injected controls. These results appear to substantiate the original hypothesis that there is a relationship between B_{12} and carbohydrate metabolism."

MILK STUDIES

601 ANNOTATION *X nutrient identified as Vitamin B_{12}*, News Edition (Chem. & Engin.) 27 1318, May 2, 1949

The hitherto unidentified food material in milk that was first reported in 1932 by the Bureau of Dairy Industry and called nutrient X because of its unknown chemical nature, is undoubtedly vitamin B_{12}, the U S. Department of Agriculture has announced.

602. CUTHBERTSON W F J., and THORNTON D M. *The effect of dietary lactose on the response of the rat to vitamin B_{12}*, Brit. Med. J 5 xli, 1951.

Marked depression of growth was observed in weanling rats fed a soya diet containing 20% lactose. This effect was completely overcome by 30 to 40 mcg. of vitamin B_{12} orally per week.

The amounts of vitamin B_{12} required to produce significant growth increments were such as to make impracticable vitamin B_{12} assay based on its growth-promoting effects for these rats.

603. COLLINS, R. A., DIETRICH, L. S., and ELVEHJEM, C. A.: *Significance of vitamin B_{12} in milk diets*, Federation Proc. 9 355, March 1950.

"The inferior growth observed in rats fed goat s milk mineralized with iron, copper and manganese as compared to the good growth of rats fed mineralized cow's milk was studied. This inferior growth was counteracted by a daily addition to the mineralized goat s milk diet of 50 µg. of folic acid plus 0.1 µg. of vitamin B_{12}, 0.5 µg. vitamin B_{12} alone, or 1.0 Gm. of fresh beef liver An inconsistent half maximal growth stimulation resulted when 50 µg. of folic acid or 0.1 µg. of vitamin B_{12} was added separately to this diet. Goat's milk was found to contain only traces of vitamin B_{12} in contrast to the occurrence of 2-3 µg. of vitamin B_{12}/L in cow s milk. The folic acid content of goat's milk was found to be very low and comparable to cow s milk. Folic acid or vitamin B_{12} additions to mineralized cow s milk diets did not affect growth in the young rat. The addition of vitamin C to the cow s milk diets produced a large increase in the vitamin B_{12} content of the livers, while on the goat s milk diets added vitamin C displayed no activity Folic acid and vitamin B_{12} additions to mineralized cow's milk or goat s milk did not affect the rate of hemoglobin formation in the weanling rat or in rats made anemic on milk diets."

604 BUREAU OF DAIRY INDUSTRY *Vitamin B_{12}-active materials in milk aid in utilization of protein*, Report of the Chief of the Bureau of Dairy Industry U S. Department of Agriculture, 1950 (cited in J Am. Dietet. A. 27 120, Feb. 1951)

"In investigations of the sources of vitamin B_{12} in milk, the Bureau of Dairy Industry U S. Department of Agriculture, found that some rats can synthesize vitamin B_{12}-active materials microbiologically when they are on rations consisting almost entirely of plant products. These results tend to support the view expressed previously which was based on strong circumstantial evidence, that the cow also is able to synthesize vitamin B_{12}-active materials which she secretes in her milk.

"Other phases of the study showed that vitamin B_{12} is effective in improving diets that are low in protein, as well as being effective in enabling rats to use rations that contain high levels of protein. Without a source of vitamin B_{12}, high levels of protein were found to be very harmful, and even fatal.

"Vitamin B_{12} was also shown to be essential for normal growth of rats when the ration was high in lactose (milk sugar) The depressing effect on growth, when the diet contains lactose but lacks vitamin B_{12}, appears to be due, in part at least, to suppression of microbiologic synthesis of this vitamin in the rat."

605. COLLINS, R. A., HARPER, A. E., SCHREIBER, M., and ELVEHJEM, C. A. *Folic acid and vitamin B_{12} content of milk of various species*, J Nutrition 43 313-321, Feb. 1951.

HYPERTHYROIDISM: VITAMIN B_{12} IN

606. NICHOL, C. A., DIETRICH, L. S., CRAVENS, W W., and ELVEHJEM, C. A.: *Activity of vitamin B_{12} in the growth of chicks*, Proc. Soc. Exper Biol. & Med. 70 40-42, Jan. 1949

Experiments are reported which indicate that vitamin B_{12} is highly active in stimulating the growth of the hyperthyroid chick. Hyperthyroidism was induced by feeding a basal ration containing 0.05% iodinated casein. Supplementation of this diet with 3% fish solubles produced in a two week period an average gain over the unsupplemented group of 86 Gm. per chick vitamin B_{12} at the level of 0.75 mcg./100 Gm. of ration resulted in a gain of 47 Gm., and 1.5 mcg./100 Gm. produced a gain of 88 Gm. The gain in weight over the unsupplemented group was 86 Gm. when reticulogen was given intravenously in the dosage of 0.5 U.S.P unit per bird per day Daily intravenous injection of 0.01 mcg. vitamin B_{12} gave a growth response of 21 Gm., 0.1 mcg. a response of 72 Gm., and 0.5 mcg. a response of 79 Gm. It is concluded that pure vitamin B_{12} can replace the animal protein factor activity of condensed fish solubles and injectable liver preparations.

607 BOSSHARDT D K., PAUL, W J., O'DOHERTY K., HUFF, J W., and BARNES R. H.: *Mouse growth assay procedures for the "animal protein factor"* J Nutrition 37 21-35, Jan. 1949

The "animal factor" has been assayed by two methods, one of which involves the use of growing mice born of mothers that were maintained on a diet free of this factor while the other is based on the ability of the "animal protein factor" to counteract the growth retardation of mice that are fed materials having thyroid activity Studies showed that liver contains a factor or factors essential for growth not present in yeast or wheat germ, and not identical with any known vitamin factor This factor counteracts the growth retardation of mice that are fed thyroid-active materials. It may be transmitted from the mother to the young during gestation or lactation or both and may be stored by the animal for a considerable length of time. A severe lack of the "animal protein factor" in the maternal diet and tissue stores results in a pronounced mortality of young mice one to three days post partum. The possibility is suggested that the "animal protein factor" may be identical with vitamin B_{12}.

608. EMERSON G.: *Growth-promoting activity of vitamin B_{12} in rats receiving thyroid substance* Proc. Soc. Exper Biol. & Med. 70 392-394, March 1949

The growth of rats on a soybean meal diet was greatly retarded by the addition of 0.25% thyroid powder The daily addition of 0.125 mcg vitamin B_{12} resulted in a weight gain exceeding that of rats receiving the soybean meal without thyroid, and approximating that of rats on a casein diet. A lower level of vitamin B_{12} reduced the gain, but higher levels did not greatly increase it. Cobalt did not increase the weight gain over that of the controls, which indicates that cobalt could not be employed under these conditions in the biosynthesis of vitamin B_{12}.

Merck Institute for Therapeutic Research
Rahway N J

609 ERSHOFF B. H.: *Beneficial effects of liver on growth and survival in the immature hyperthyroid mouse*, Proc. Soc. Exper Biol. & Med. 70 398-401 March 1949

Whole liver powder prolonged survival and counteracted the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin, or iodinated casein. The protective factor(s) was apparently distinct from any of the known nutrients and was retained in the water-insoluble extracted liver residue.

Contrary to the findings of Bosshardt *et al.* (Abstr 607) Wilson s liver concentrate 1 20 did not increase the growth or survival of mice fed iodinated casein or desiccated thyroid, but did promote the growth of mice fed thyroxin although it was less effective in this respect than extracted liver residue. Since liver concentrate 1 20 contains vitamin B_{12}, factors other than this vitamin apparently are required by the hyperthyroid mouse. A multiple dietary deficiency may be precipitated in animals on purified rations when they are made hyperthyroid. Whether the first limiting factor is a deficiency of vitamin B_{12}, or the antithyrotoxic factor(s) of extracted liver residue or of still other nutrients appears to depend on such factors as the pre-test dietary regimen, the composition of the diet employed, bacterial synthesis, or strain and species differences in nutritional requirements.

610. BETHEIL, J J., and LARDY H. A.: *Comparative effectiveness of vitamin B_{12}, whole liver substance and extracts high in APA activity as growth promoting materials for hyperthyroid animals*, J Nutrition 37 495-509 April 1949

In thyrotoxicosis, rats require one or more growth factors which are not needed by normal rats fed highly purified diets. Purified liver extracts rich in the anti pernicious anemia (APA) factor are a good source of this growth factor(s) The growth response may be due to some constituent other than the APA factor since the thyrotoxicosis produced by the thyroid-containing ration is not accompanied by an anemia and the hemoglobin levels of animals showing the typical growth depression are normal. However on a dry weight basis liver fractions containing less APA activity are also less active in supporting the growth of hyperthyroid rats. If this growth stimulating factor is the same as that required for blood cell maturation in human beings, these observations may serve as the basis for an animal assay for the APA factor The graded growth response obtained with increasing doses of purified liver extracts indicates the possibility of such an assay

The effect of APA preparations was also tried in rabbits. During a six week period of thyroxine administration, 6 of 7 control animals died, while 6 of 7 that were given purified liver extract for the last four weeks of the period survived although they did not grow

The intraperitoneal administration to hyperthyroid rats of 0.2 mcg. crystalline vitamin B_{12} daily or the addition of 30 mcg. B_{12} per Kg of ration, promoted growth as well as did the liver extract. Thus it seems that all or most of the growth-promoting activity of APA liver extracts resides in their vitamin B_{12} content. It appears that the vitamin B_{12} requirement of the hyperthyroid rat lies between approximately 0.1 and 0.2 mcg. per day when the vitamin is administered parenterally

Exhaustive extraction of whole liver powder with ethanol, ethanol-ether or water brought only part of the active material into solution, which is another indication of the possible dual nature of the material which, in addition to the known vitamins, supports the growth of animals fed thyroxine-containing rations.

Treatment of folic acid with good sources of the "intrinsic factor" did not result in the formation of a growth-promoting substance, and "formylfolic acid" was likewise ineffective.

611 ERSHOFF B. H.: *An antithyrotoxic factor for the rat not identified with vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 71 209-211, June 1949

On purified rations containing casein as the dietary protein and sucrose as the dietary carbohydrate, the growth of hyperthyroid rats was reduced. The retardation of growth was completely counteracted by the administration of a water insoluble fraction of liver but crystalline vitamin B_{12} was ineffective. The protective factor in liver is distinct from any of the known nutrients, including vitamin B_{12}.

612. ERSHOFF B. H. *Protective effects of soybean meal for the immature hyperthyroid rat*, J Nutrition 39 259-281, Oct. 1949

Full-fat soybean meal completely counteracted the growth retardation of immature rats fed massive doses of desiccated thyroid or iodinated casein. The retardation in growth of immature hyperthyroid rats on a diet containing low-fat soybean flour was completely counteracted by the administration of either soybean oil, extracted liver residue or crystalline vitamin B_{12}. When immature rats were fed a similar diet but containing casein as the dietary protein, the growth retardation following thyroid feeding was completely counteracted only by extracted liver residue, vitamin B_{12} being inactive.

University of Southern California
Los Angeles, Calif.

613 ERSHOFF B. H. *Effects of vitamin B_{12} and liver residue on growth of hyperthyroid male rats*, Proc. Soc. Exper Biol. & Med. 73 459-461, March 1950.

Massive doses of thyroid in conjunction with a purified diet containing casein as the dietary protein resulted in retardation in both body and gonadal weight in immature male rats. These effects were completely counteracted by the administration of liver residue. Crystalline vitamin B_{12}, however at a level of 30 mcg. per Kg. of diet, was without significant effect. The growth retardation and inhibition of testicular development is apparently due to a deficiency of some factor other than vitamin B_{12}.

University of Southern California
Los Angeles, Calif

614 LEWIS U J., TAPPAN D V., REGISTER, U D., and ELVEHJEM, C. A. *Effect of carbohydrate on growth response to vitamin B_{12} in the hyperthyroid rat*, Proc. Soc. Exper Biol. & Med. 74 568-571 July 1950.

Studies were undertaken to elucidate the nature of antithyrotoxic effects of various carbohydrates in relation to the specificity of the rat assay for vitamin B_{12}. Rats on a sucrose-casein diet containing a thyroid-active material do not respond completely to vitamin B_{12}, but this can be overcome by substituting defatted corn meal, corn starch or dextrin for the sucrose. This may possibly be attributed to the intestinal synthesis of other required factors.

Growth depression in the hyperthyroid rat was counteracted to a slight extent by the addition of corn oil to the corn-soybean ration containing iodinated casein, but this may have been due to the fact that these animals consumed less food and, therefore, less iodinated casein.

University of Wisconsin
Madison, Wis.

615. PENTZ, E. I., GRAHAM, C. E., RYAN, D E., and KLEIN, D *The ability of liver preparations and vitamin B_{12} to maintain thymus weight in thyroid fed rats having greatly hypertrophied adrenal glands*, Endocrinology 47 80-85, July 1950.

A study is described in which it was found that vitamin B_{12} has a protective effect on the thymus in rats fed thyroid. Rats bred on a ration containing soybean meal, dextrose, and vegetable fat, supplemented with excess amounts of all the crystalline vitamins, were fed this diet plus 0.25 per cent desiccated thyroid with and without supplements of injectable liver preparations, a vitamin B_{12} concentrate and crystalline vitamin B_{12}.

Autopsy studies revealed that the rats receiving desiccated thyroid alone had greatly enlarged adrenal glands and atrophy of the thymus. Rats which received liver preparations, the vitamin B_{12} concentrate, and crystalline vitamin B_{12} in addition to the desiccated thyroid, had equally large adrenals but did not show atrophy of the thymus. One of the functions of vitamin B_{12} in bacterial metabolism is in the synthesis of the thymine desoxyriboside. It has been shown previously that the two areas containing the highest concentration of newly formed desoxyribonucleic acids are the thymus and the crypts of Lieberkuhn. This may provide the basis for an explanation of the protective effect of vitamin B_{12} on thymus weight in the present experiments.

The Wilson Laboratories
Chicago, Ill.

616. MEITES, J *Effects of vitamin B_{12} on thiouracil action in rats* Proc. Soc. Exper Biol. & Med. 75 193-195, Oct. 1950.

Author's summary "The effects of crystalline vit. B_{12} on thiouracil action was determined in immature female rats for a 30-day period. The vitamin completely counteracted the growth inhibiting action of thiouracil, and this was accompanied by a considerable increase in food consumption. Although vit. B_{12} decreased the thyroid hypertrophy induced by thiouracil, the uptake of radioactive iodine (I^{131}) by the thyroids was even less than in the rats which received thiouracil only It is suggested that vitamin B_{12} may be able to induce normal growth in hypothyroid rats."

Michigan State College
East Lansing, Mich.

617 MEITES, J: *Effects of vitamin B_{12} on normal thyroid function in rats*, Proc. Soc. Exper Biol. & Med. 75 195-197 Oct. 1950.

Author's summary: "Crystalline vit. B_{12} was fed to immature rats of both sexes in order to determine whether the vitamin could alter thyroid function in normal or thyroprotein-treated rats. The growth rate of the rats supplemented with the vitamin was increased above that of the normal or thyroprotein-treated controls, but there was no significant effect on thyroid weight or uptake of I^{131} It is concluded that vit. B_{12} does not alter normal thyroid activity in rats."

Michigan State College
East Lansing, Mich.

618. BOLENE, C., ROSS, O B., and MacVICAR, R.: *The growth promoting action of various supplements in the hyperthyroid rat*, Proc. Soc. Exper Biol. & Med. 75 610-613, Nov 1950.

The reversal of growth inhibition induced by feeding thyroid-active substances to the immature rat has been used by other workers as an assay procedure. In the experiments reported here, rats receiving toxic levels of iodinated casein were given various supplements to counteract the effect of the iodinated casein on growth.

Fish solubles, 1.5 per cent in the diet, two commercial APF supplements and 1:20 liver powder all of which have appreciable animal protein factor activity for the chick, proved effective in counteracting the inhibition of growth by iodinated casein. Crystalline vitamin B_{12} gave a highly significant growth response in males, but was less effective in females. Even in males it did not produce maximum response. This suggests that the "antithyrotoxic factor" is multiple in nature and that vitamin B_{12} is one of the components.

Oklahoma Agricultural and Mechanical College
Stillwater Okla.

619 RUPP J., PASCHKIS, K. E., and CANTAROW A.: *Influence of vitamin B_{12} and liver extract on nitrogen balance of normal and hyperthyroid rats*, Proc. Soc. Exper Biol. & Med. 76 432-435, March 1951

Vitamin B_{12} (3 mcg twice daily) or liver extract (0.2 cc.) did not induce gain in weight nor nitrogen retention in force-fed rats on constant food intake. Injection of thyroxine produced identical weight losses in the rats given vitamin B_{12} and in the controls, although the nitrogen loss resulting from the catabolic action of thyroxine was significantly less in the treated animals. Liver extract had a similar action, but the values obtained were not statistically significant. These results suggest that in hyperthyroid rats, vitamin B_{12} spares protein at the expense of other body constituents.

Sloan-Kettering Institute for Cancer Research
New York, N Y

620. RUPP J *Action of vitamin B_{12} and of liver extract on nitrogen balance of hyperthyroid rats* Federation Proc. 10 115, March 1951.

"Considerable experimental evidence has accumulated during the past several years showing that the weight loss of hyperthyroid animals can be prevented, and even normal growth of such animals maintained, if vitamin B_{12} or liver preparations are administered. This effect is largely due to stimulation of appetite induced by the supplements, but it has been suggested that in addition food utilization is improved. We have performed experiments on force-fed rats fed a constant amount of a diet containing about 16% protein derived from yeast and milk powder Neither vitamin B_{12} (6 γ/day) nor liver extract (0.4 U.S.P U — 0.2 ml./day) caused any change of weight or of N excretion. Rats fed in this way and made hyperthyroid by daily injection of 150 γ of *d-l* thyroxine, lost weight and exhibited increased N excretion. When such thyroxine-treated rats were given vitamin B_{12}, the thyroxine induced weight loss was not changed, but the N loss was significantly decreased. Since it is known that the calorigenic effect of thyroxine is not counteracted by the vitamin and since the weight loss of the hyperthyroid rat was not influenced by B_{12} or liver extract, it would appear that the latter are N sparing at the expense of material other than protein."

Jefferson Medical College
Philadelphia Pa.

621 MEITES J *Counteraction of anabolic effects of mild hyperthyroidism by vitamin B_{12}*, Federation Proc. 10 91 March 1951.

"Vitamin B_{12} has been shown to counteract the growth-retarding effects induced in rats by administering thyroid-active materials. Young mice, unlike rats, respond to the administration of low doses of thyroid substances by true increases in growth rate, and it was of interest to determine whether vitamin B_{12} could modify this effect. Forty immature albino mice (Rockland) weighing approximately 13.5 gm. each, were divided into 4 uniform groups and fed a basal ration low in vitamin B_{12} for 30 days. The following substances were added to the ration of each group 1) controls, nothing 2) 0.025% thyroprotein (Cerophyl) 3) 100 µg. of vitamin B_{12} (Merck) per kg. of ration; 4) 0.025% thyroprotein and 100 µg. of vitamin B_{12}. The total average gain in body weight and daily average food consumption in grams per mouse were as follows for each group 1) 9.6 and 3.2 2) 18.5 and 5.4 3) 15.9 and 4.0 4) 10 6 and 4.0. It can be seen that either thyroprotein or vitamin B_{12} alone considerably increased body weight and food intake above the controls, while the combination of the two substances eliminated the favorable effects of either on body growth. The efficiency in converting food into body gains was greatest for the groups receiving thyroprotein or vitamin B_{12} alone, and least for the group receiving both substances."

Michigan State College
East Lansing, Mich.

622. GREER, M. A. *Failure of vitamin B_{12} to modify goitrogenic action of thiouracil*, Proc. Soc. Exper Biol. & Med. 77 146-147 May 1951

The development of goiters in rats fed thiouracil was not affected by the addition to the diet of an aqueous solution of vitamin B_{12} or of crystalline B_{12} as a triturate of sodium chloride. This is contrary to the findings reported by Meites (*Proc. Soc. Exper Biol. & Med* 75 193, 1950 [Abstr 616])

New England Center Hospital, and
Tufts College Medical School
Boston, Mass.

623 WATTS, A. B., ROSS, O B., WHITEHAIR, C. K., and MacVICAR, R. *Response of castrated male and female hyperthyroid rats to vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 77 621-626, Aug. 1951.

Because of conflicting reports regarding the influence of sex on response to vitamin B_{12}, the growth promoting effect of this vitamin in the hyperthyroid rat was studied in normal and castrate rats of both sexes. Growth depression was produced by feeding toxic levels of iodinated casein. Vitamin B_{12}, 25 mg. per Kg., was less effective than liver concentrate powder 1:20, in counteracting growth depression in normal males and in oophorectomized females. There was little difference in response to these supplements in normal females and castrate males. It is suggested that the male sex hormone may act synergistically in the hyperthyroid rat with some factor present in liver and not identical with vitamin B_{12}, or that the female sex hormone is antagonistic to such a factor

Oklahoma Agricultural Experiment Station
Stillwater Okla.

624 DRYSDALE, G. R., BETHEIL, J J., LARDY H. A., and BAUMANN C. A. *The excretion of the citrovorum factor by hyperthyroid rats*, Arch. Biochem. & Biophysics 33 1-8, Aug. 1951.

In immature rats fed diets containing folic acid and 0.25% of desiccated thyroid, the administration of vitamin B_{12} or of whole liver powder caused an increased urinary excretion of the citrovorum factor

625. BARKER, S. B., DIRKS, H. B., JR., KLITGAARD H. M., and GARLICK, W R. *Inhibition of metabolic action of thyroxine*, Am. J Physiol. 167 765, Dec. 1951 (in Soc. Proc.)

"Using the thyroidectomized rat maintained on 12 µg. DL-thyroxine as a test animal, we have continued the investigation of the capacity of substances to interfere with the metabolic action of the hormone. Molecular ratios of compound to thyroxine have been computed on the basis of the quantities injected, assuming no endogenous source of thyroxine. Synthetic vitamin A, both as the solution in oil and saline suspension of the palmitate, exhibited inhibiting effects of 20-40% at molar ratios of 300-2000. Folic acid was inactive at 500x, but B_{12} showed a 21% effect at 10x. The 5-iodothiouracil and 3-fluoro-4-hydroxyphenylacetic acid both depressed thyroxine action about 40% at doses 500x isomolar to that of the hormone. Several members of a series of organic acids containing a 3,5-diiodo-4-hydroxyphenyl substitution displayed thyroxine-inhibiting qualities. Various derivatives of 3,5-diiodo-4-hydroxybenzoic acid also were active antagonists, although 3,5-diiodotyrosine exhibited some thyroxine-like action."

State University of Iowa
Iowa City Iowa

626. ARCHER, J D., NASH, J B., BROWN R. G., and EMERSON G A. *Failure of excess vitamin B_{12} in influencing metabolic and toxic effects of thyroid U.S.P XIV* Federation Proc. 11 318, March 1952.

"Effects of vitamin B_{12} on viability and growth of thyroid- or thyroxin-treated rats deficient in this vitamin have been noted by several authors none describes actions of vitamin B_{12} in modifying metabolic effects of large doses of thyroid substance in rats on a relatively adequate diet. Forty five young female rats were divided into 3 groups of ca. the same mean body weights, within 3% and maintained on Purina dog chow Thyroid was given intragastrically for 16 days to 2 groups, as a suspension of the finely powdered drug, in a dose of 450 mg/kg one of these groups also received 16 daily doses of 75 µg./kg. of vitamin B_{12}, intraperitoneally O_2 consumption, estimated manometrically and body weights were noted during the last 8 days of treatment. No significant differences in either metabolic rate or weight were noted between the 2 groups receiving thyroid, but there was a substantially increased metabolic rate and a failure of growth in both treated groups as compared with the untreated controls. Similar results were found with thyroxin."

University of Texas Medical Branch
Galveston, Texas

627 GRAHAM, C. E., REICHSTEIN L P., WATSON W J., and HIER, E. W *Effects of liver fractions and vitamin B_{12} on body organ weights of thyroid fed rats*, Proc. Soc. Exper Biol. & Med. 80 657 659 Aug.-Sept. 1952.

In experiments designed to show the effect of thyroid, defatted liver residue, liver concentrate N F., and vitamin B_{12} on organ weights of young rats, vitamin B_{12} was without effect except in preventing hypertrophy of the spleen due to thyroid feeding. Lyxoflavin was found to have no antithyrotoxic effect in the diet used.

University of Rochester School of Medicine and Dentistry
Rochester N Y

628. PALMER, J G., CARTWRIGHT G E., and WINTROBE, M. M.: *Experimental production of leukopenia in rats by thyroid feeding*, Am. J Physiol. 171: 585-590, Nov 1952.

Authors summary: "Rats fed desiccated thyroid and a diet limited in caloric value developed severe leukopenia and lost weight. The leukopenia involved both granulocytes and mononuclear cells and was not accompanied by anemia. It was not corrected by increasing any one of the constitutents of the diet nor by giving vitamin B_{12}, folic acid, citrovorum factor ascorbic acid, lyxoflavin, whole liver powder vegetable fat or yeast. Evidence is presented which suggests that the leukopenia is related to a limited caloric intake."

University of Utah College of Medicine
Salt Lake City Utah

629 OVERBY L. R., FREDERICKSON R. L., and FROST D V *The antithyrotoxic factor* Federation Proc. 12 425 March 1953

"A water-insoluble liver fraction has been found by Ershoff *(Proc. Soc. Exp. Biol. and Med.* 64 500, 1947) to counteract the growth retardation of immature rats fed massive doses of thyroxin. The present study concerns assay for this factor using procedures based on the work of Ershoff and the assay in rats for vitamin B_{12} in this laboratory Responses to vitamin B_{12} or to liver residue differ depending on the level of thyroxin fed. A synthetic type sucrose, casein diet containing vitamin B_{12} was developed. The most critical assay for the antithyrotoxic factor was obtained with 0.3% iodinated casein as the thyrotoxic agent. Weanling male rats were depleted for 10 to 12 days on the basal ration without thyroxin. Maintenance of a near normal growth rate and decrease in mortality were taken as criteria of the anti-thyrotoxic activity of supplements to the basal diet. Liver residue (5-10%) partially counteracts the thyrotoxic effects. Other substances studied were synthetic and natural fats, fibrin, hydrolyzed fibrin, egg albumin, defatted whole egg, lecithin, lyxoflavin, lipoic acid, ACTH, orotic acid, adenine and guanine. Of these, only fats and defatted whole egg showed protective action against thyrotoxicosis. Isolation of the active principle in liver residue is under study "

Abbott Laboratories
North Chicago, Ill.

OTHER HORMONES

630. MEITES, J., and NEWLAND H. W: *Effects of vitamin B and thyroprotein on growth-inhibiting action of diethylstilbestrol in rats* Federation Proc. 9 87-88, March 1950.

"Growth depression induced by diethylstilbestrol in rats has been accounted for on the basis of reduced appe-

tite (Meites, *Am. J Physiol.* 159 281 1949) It was hypothesized that these inhibitory effects were induced by increasing certain vitamin needs and/or decreasing thyroid secretion. This hypothesis was tested by treating 5 evenly divided groups of 50 male rats for 20 days. Diethylstilbestrol and thyroprotein were fed in a commercial ration, while B_{12} was injected subcutaneously at a level of 0.2 mg daily During the first 10 days, B_{12} failed to counteract the growth depression induced by diethylstilbestrol, but partially overcame the effects of the latter during the last 10 days. Thyroprotein plus diethylstilbestrol-thyroprotein combination completely counteracted the growth-inhibiting action of the latter and only slightly that of the former Food intake was increased both by B_{12} and thyroprotein."

Michigan State College
East Lansing, Mich.

631. MEITES, J., and SHAY J C. *Effects of vitamin B_{12} on diethylstilbestrol and thyroprotein action in male rats,* Proc. Soc. Exper Biol. & Med. 76 196-198, Jan. 1951

The effects of crystalline vitamin B_{12} on several actions of diethylstilbestrol and thyroprotein were determined in 100 young male albino rats. Diethylstilbestrol and thyroprotein were incorporated into the ration in amounts of 0.10 and 0.16 per cent, respectively and vitamin B_{12} was injected subcutaneously in doses of 0.2 or 0.4 mcg. daily Vitamin B_{12} partially counteracted the growth-retarding action of these two hormones when given separately or together and also increased food consumption. The vitamin did not alter the ability of the hormones to depress testes weight or the ability of diethylstilbestrol to increase the weight of the seminal vesicles.

An addendum states that data obtained since the submission of this manuscript indicate that vitamin B_{12} probably does not alter the turnover of thyroprotein in the rat. The increase in the basal metabolism of rats given thyroprotein was not affected by the administration of vitamin B_{12}.

Michigan State College
East Lansing, Mich.

632. MEITES, J *Changes in nutritional requirements accompanying marked changes in hormone levels,* Metabolism 1 58-67 Jan. 1952

Author's summary "Large doses of diethylstilbestrol or thyroprotein, fed to young male rats on an adequate ration, severely inhibited body growth. Supplementation of the ration with vitamin B_{12} partially counteracted growth inhibition and increased appetite. The decrease in testes weight which resulted from the hyperestrinism or hyperthyroidism, and the increase in weight of the seminal vesicles by the estrogen, were not influenced by vitamin B_{12}.

"The inhibition of body growth in young female rats by thyroprotein was completely counteracted by incorporation of small amounts of procaine penicillin G or neomycin sulfate in an otherwise adequate diet. Streptomycin sulfate was ineffective in this respect. The large increase in basal oxygen consumption (74%) induced by thyroprotein was not affected by the antibiotics.

"Of 18 young male rats on a vitamin B_{12}-deficient ration subjected to thyroparathyroidectomy 15 survived for 30 days, 5 for 60 days, and none for 90 days. When the diet was supplemented with vitamin B_{12}, 21 out of 26 survived for 30 days, 19 survived for 60 days, 17 for 90 days, and 16 for 120 days. The vitamin enabled growth to proceed in the surviving rats, although at a considerably slower rate than in intact rats given vitamin B_{12}.

"Large daily injections of cortisone inhibited body and hair growth in young male rats fed a ration deficient in vitamin B_{12}. The addition of vitamin B_{12}, either alone or together with aureomycin hydrochloride, to the diet completely counteracted inhibition of body growth and partially of hair growth. The vitamin and antibiotic completely protected the thymus against atrophy by cortisone but did not prevent the decrease in adrenal weight.

"It is concluded that (a) marked changes in thyroid, estrogen, or cortisone levels in the body can increase requirements for vitamin B_{12} and possibly other dietary factors in rats (b) supplementation of diets with vitamin B_{12} and antibiotics can partially or completely prevent manifestations of dietary deficiencies induced by these hormones and (c) vitamin B_{12} and the antibiotics do not alter certain characteristic actions of these hormones in the body with the exception that cortisone-induced atrophy of the thymus can be prevented by vitamin B_{12} and aureomycin."

Michigan State College
East Lansing, Mich.

633 DACOSTA, E., and CLAYTON R. *Histological and biochemical changes in the rat adrenal after dietary restriction and rehabilitation,* Am. J *Physiol.* 171 717 Dec. 1952 (in Soc. Proc.)

"The weight, total cholesterol, vitamin C, sudanophilic and birefringent material of the adrenal and eosinophil count were determined on 288 rats after 35 days of either *1)* caloric restriction, *2)* a 2% protein diet, *3)* a 4.5 protein and 10% salt diet and after 35 days of rehabilitation on either *4)* a 60% protein ad libitum, *5)* the same, 11 gms./day or *6)* the same plus B_{12}. In the calorically restricted or high protein controls less birefringence occurred in all zones. Increased sudanophilic or birefringent material was found in the fasciculata after *diets* 2 and 3. During rehabilitation after caloric restriction sudanophilic and birefringent materials decreased in the reticulata and increased in the glomerulosa. During rehabilitation after *diet* 2, the sudanophilic distribution resembled the control while birefringence increased in the fasciculata. Following rehabilitation after *diet* 3, sudanophilic and birefringent material was decreased in the glomerulosa but increased in the fasciculata and reticulata. Usually a *reciprocal* relationship existed between adrenal weight and number of eosinophils and between vitamin C and cholesterol content. The adrenals of the high protein controls weighed 50.2 mg., eosinophils 0.55% of total white cells, cholesterol 0.65 mg. % vitamin C 0.24 mg. % compared to 45.2 mg., 1 09% 2.4 mg. % and 0.18 mg. % for the normal controls and 24.0 mg., 1.51% 10 and 0.07 mg. % after diet 5 respectively Thus restriction and rehabilitation evidently involve adaptation partly controlled by the adrenals."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

634 GERSHBERG H., and RALLI, E. P *Adrenal changes associated with a low protein diet*, Federation Proc. 11 54, March 1952.

"It is established that a low protein–high fat diet will produce fatty infiltration of the liver Other tissues probably also suffer from this protein deficiency in particular the pituitary which secretes protein hormones. In rats fed such a diet (9% protein and 38% fat) the adrenals and the thymus were weighed and determinations were made of the adrenal cholesterol, liver fat and liver nitrogens after periods of 8 to 60 days on the diet. The adrenal cholesterol was increased about 2 g.% after only 8 to 12 days on the diet. After 54 days, the adrenal cholesterol was further elevated and the adrenal weight had diminished. These changes are in the same direction as those observed in hypophysectomized rats, though not as pronounced. The increase in adrenal cholesterol was not associated with an increase in liver brain or serum cholesterol which in most cases was within normal limits. Administration of ACTH (2 mg daily) caused an increase in adrenal weight and a decrease in thymus weight, indicating that the adrenals were not refractory Vitamin B_{12} (5 mcg. daily) had a distinct lipotropic effect on the liver within a period of 7 to 12 days, but the adrenal cholesterol was not reduced."

University of New York College of Medicine, and Bellevue Medical Center New York, N. Y

635. RUPP J., and PASCHKIS, K. E.: *Lack of influence of vitamin B_{12} on protein catabolic action of cortisone*, Proc. Soc. Exper Biol. & Med. 82 65-66, Jan. 1953.

Authors summary "In rats receiving a constant food intake by tube feeding, the weight loss and N loss induced by cortisone is not improved by treatment with vitamin B_{12}. This failure of vitamin B_{12} to influence cortisone induced protein catabolism is in contradistinction to the N sparing effect of vitamin B_{12} in thyroxine induced protein catabolism.

Jefferson Medical College Philadelphia, Pa.

ANTIBIOTIC SUPPLEMENTS

Chicks

636. STOKSTAD E. L. R., and JUKES, T H. *Further observations on the "animal protein factor"* Proc. Soc. Exper Biol. & Med. 73 523-528, March 1950.

Chicks with a low reserve of the animal protein factor were kept on experimental diets adequately supplied with vitamin B_{12}, either in the crystalline form or as a mixture of vitamin B_{12} and vitamin B_{12b} containing more than 10 mcg. of the mixed vitamins per milligram of organic solids. Growth was promoted by two types of aureomycin fermentation products—a dry mixture of the mycelium plus a variable amount of filter-aid, and a concentrate of the whole fermented mash from which water had been removed by evaporation. Growth responses were also produced by crystalline aureomycin hydrochloride and by cultures of Streptomycin aureofaciens in which the aureomycin had been destroyed by alkaline hydrolysis. Responses were also obtained with succinylsulfathiazole, streptomycin and 3-nitro-4-hydroxyphenylarsonic acid, but these substances appeared less potent than aureomycin. No responses were obtained with dried whey or dried brewer's yeast. The fact that growth responses have been obtained by such widely differing types of compounds supports the view that the growth produced by aureomycin is related to its antibiotic activity

An addendum reports a manuscript shown the authors after submission of this article, in which Gyorgy Stokes, Smith and Goldblatt *(Tr of Conf on Biol. Antioxidants*, sponsored by the Macy Foundation, Nov 1949 *see also* Abstr 747) described a beneficial effect exerted by aureomycin on growth and prevention of dietary hepatic necrosis in rats. The assumption was made that aureomycin might act by suppressing the intestinal flora.

Lederle Laboratories Pearl River N Y

637 WHITEHILL, A. R., OLESON J J., and HUTCHINGS, B. L. *Stimulatory effect of aureomycin on the growth of chicks*, Proc. Soc. Exper Biol. & Med. 74 11 13, May 1950.

On the basis of findings of other investigators that for chicks the growth-promoting activity of "animal protein factor" concentrates is not due to their B_{12} content alone, and that Streptomyces aureofaciens fermentation liquors (of which aureomycin is a constituent) have increased growth, the growth-promoting effect of aureomycin, and certain other antibiotics were added to three test diets. All diets were supplemented with vitamin B_{12} 90 mcg./kg.

Results indicate that the combined diet effected greater growth stimulation than that produced by vitamin B_{12} alone. Of the other antibiotics given at the level of 100 mg./kg., only penicillin produced a response equivalent to that produced by aureomycin. The effect of aureomycin is presumed to be indirect, since certain other antibiotics manifest the same property

Lederle Laboratories Pearl River N Y

638. OLESON J J., HUTCHINGS, B. L., and WHITEHILL, A. R. *The effect of feeding aureomycin on the vitamin B_{12} requirement of the chick*, Arch. Biochem. 29 334-338, Dec. 1950.

The interdependence of the growth effects of vitamin B_{12} and aureomycin in the chick were studied. Each factor appears to have a sparing action on the other The effect of aureomycin on the growth of chicks fed no vitamin B_{12} supplement and fed an excess of vitamin B_{12} was determined. In the absence of vitamin B_{12} no stimulation was obtained, while a definite growth increase was evident when vitamin B_{12} was present. When chicks were fed sufficient levels of vitamin B_{12} to give suboptimal and optimal growth responses, a marked weight increase was obtained by the addition of aureomycin.

An attempt was made to determine the minimal vitamin B_{12} level essential to demonstrate the aureomycin growth effect; it appears that a level somewhere between 2.1 and 4.2 mcg. per Kg. of diet is required. From this level to 90 mcg. per Kg. of diet the antibiotic stimulatory effect was evident. Aureomycin gives growth stimulation at levels as low as 5 mg. per Kg. of diet.

Preliminary experiments with rats are described which show that several antibiotics (aureomycin, penicillin, streptomycin, and chloramphenicol) are capable of stimulating the growth of this species. The growth-stimulating effect of the antibiotics appears to be an indirect mechanism and not dependent upon the structure of the antibiotic.

Lederle Laboratories
Pearl River N Y

639 BERG L. R., BEARSE, G E., McGINNIS, J., and MILLER, V L.: *The effect of removing supplemental aureomycin from the ration on the subsequent growth of chicks*, Arch. Biochem. 29 404-407 Dec. 1950.

Chicks continued to gain weight when B_{12} was dropped from the diet at 4 weeks. Deletion of aureomycin from the ration resulted in a cessation of the accelerated rate of gain observed prior to the test.

640. COATES, M. E., HARRISON G. F., KON S. K., MANN M. E., and ROSE, C. D.: *Effect of antibiotics and vitamin B_{12} on the growth of normal and animal protein factor deficient chicks* Biochem. J 48 xii, Jan. 1951 (in Proc. Biochem. Soc.)

641. STOKSTAD E. L. R., and JUKES, T H.: *Effect of various levels of vitamin B_{12} upon growth response produced by aureomycin in chicks*, Proc. Soc. Exper Biol. & Med. 76 73-76, Jan. 1951

Chicks on a vitamin B_{12}-deficient diet were fed graded levels of vitamin B_{12} in the presence and absence of aureomycin. In some experiments aureomycin seemed to have a sparing effect on vitamin B_{12} requirement, but in others no effect was observed. Aureomycin promoted growth both in the presence and absence of vitamin B_{12} supplements. The mortality of vitamin B_{12}-deficient chicks on the diet containing no vitamin B_{12} was reduced by aureomycin. The addition of sulfamethazine to the diet did not appear to affect the quantitative requirement of chicks for vitamin B_{12}.

This may be due to differences in the intestinal flora.

Lederle Laboratories
Pearl River N Y

642. ATKINSON R. L., and COUCH, J R.: *Vitamin B_{12}, an APF concentrate, aureomycin, streptomycin, liver "L" and fish meal and fish solubles in the nutrition of the poult*, J Nutrition 44 249-263, June 1951

The addition of 66 mg. of streptomycin plus vitamin B_{12} 13 mcg. per Kg of a corn-soybean oil meal ration containing bone meal, oyster shell, salt, manganese sulfate fortified fish oil, vitamin D and riboflavin provided for approximately 15 per cent increase in the growth rate of poults at 8 weeks of age. Similar increases were observed in birds receiving 2 per cent APF No. 4 (B_{12} 12 mcg./Kg.)

The injection of 2 mcg. of vitamin B_{12} per bird per week had no effect on growth and feed efficiency The substitution of 4% liver "L" (B_{12} 49 4 mcg /Kg.) for an equivalent amount of soybean oil meal in the diet produced birds that weighed more than those fed 6% fish meal and 3% fish solubles. The liver "L" fraction also improved feed efficiency

Mortality was higher in the groups receiving the B_{12}-streptomycin supplement (10%) or the fish meal-fish solubles (16%) A 5% mortality was observed in groups fed the APF concentrate, while only 2% mortality occurred in those given the B_{12} injections, liver "L" or the unsupplemented basal.

In a second trial, eight groups of 45 birds each were fed the same corn-soybean oil meal ration used in the first experiment plus the following added quantities of B-vitamins in milligrams per Kg.: thiamine 4, riboflavin 6, calcium pantothenate 15, niacin 100, pyridoxine 4, folic acid 2, biotin 0.2, and choline 2,000. Significant increases in growth rate were obtained by supplementing this basal with Merck MK 23 (streptomycin 66 mg. plus B_{12} 13 mcg./Kg.) aureomycin 100 mg./Kg. alone or in combination with 2 mcg. of B_{12} injected per bird per week, 6% fish meal plus 3% fish solubles, or 4% liver "L" (B_{12} 49.6 mcg./Kg.) The greatest growth response of approximately 11% was obtained on the aureomycin B_{12} fortified diet. Birds on the basal alone weighed more than did those injected with 2 mcg. of B_{12} per week.

Poults on the aureomycin B_{12}, liver "L" and B_{12} (injected) supplemented diets were continued on test for an additional six weeks to determine whether the weight increases which were apparent at eight weeks would continue to persist. The aureomycin-B_{12} and injected B_{12} continued to accelerate the growth of the turkey poults up to and including the fourteenth week of the growth period.

When a comparison was made between the weights of birds on the corn-soybean basal and those on the B-vitamin fortified basal similarly supplemented, it was found that the 8 crystalline B-vitamins promoted the growth of poults in some instances but failed to have an appreciable effect in the presence of streptomycin and B_{12} or when 2% APF (B_{12} 12 mcg./Kg.) supplements were fed. Feed efficiency and mortality data were also given for the second trial.

Several possibilities were discussed as to the mode of action of the antibiotics in stimulating poult growth.

Agricultural and Mechanical College of Texas
College Station, Texas

643. ELAM, J E., GEE, L. L., and COUCH, J R.: *Function and metabolic significance of penicillin and bacitracin in the chick*, Proc. Soc. Exper Biol. & Med. 78 832-836, Dec. 1951.

The effect of oral and parenteral administration of penicillin, bacitracin, and autoclaved penicillin on growth and aerobic fecal microflora of chicks was studied. The New Hampshire chicks used in these trials were obtained from hens fed a corn-milo-soybean oil meal basal ration fortified with fish meal, an APF-B_{12} concentrate, vitamins and minerals. Sixteen groups of 30 chicks each were used and were kept in batteries with raised screen floors for a 10-week experimental period.

The administration of oral or injected penic- (amounts employed not given) increased the rate

growth. A slightly greater growth increase was obtained when penicillin in sesame oil (containing aluminum monostearate) and autoclaved penicillin were each injected.

The injection of vitamin B_{12} resulted in an increase in growth. When both B_{12} and penicillin were given an additional growth response was produced.

Bacitracin given orally or parenterally increased growth over that obtained on the unsupplemented basal this increase was of the same magnitude as that produced by adding B_{12} alone to the diet. Birds receiving bacitracin orally or parenterally with B_{12} weighed more at the end of the 10-week test period than did those of any other group.

The oral administration of penicillin produced an increase in the penicillin and aureomycin-resistant organisms and in yeast counts. There was no significant increase in the total dilution count, enterococci, lactics, or coliforms in the feces of chicks receiving oral penicillin or the unsupplemented basal. Penicillin given parenterally had no effect on the aerobic fecal microflora. When penicillin was administered in sesame oil containing aluminum monostearate, there was an increase in the penicillin-resistant organisms and in the yeast counts.

Bacitracin given orally or parenterally with or without B_{12}, had no effect on the thioglycollate count, enterococci, lactics, yeasts, coliforms, or penicillin or bacitracin-resistant organisms.

The results obtained in these experiments lead the authors to believe that the antibiotic molecule or a fragment thereof might act as a metabolite within the body of the bird.

Agricultural and Mechanical College of Texas
College Station, Texas

644 LIBBY D., and MEITES J *Effects of vitamin B_{12} and penicillin on thiouracil action in chicks*, Proc. Soc. Exper Biol. & Med. 79 370-372, March 1952.

The effect of vitamin B_{12} and penicillin, both of which have been shown to stimulate chick growth, was studied in a total of 168 thiouracil-treated chicks.

Authors summary "1. White Leghorn male and Rhode Island Red chicks of both sexes were fed a vit. B_{12}-deficient ration which was supplemented with 0 1% thiouracil and/or 0.5 to 4.0 µg. % vit. B_{12} or 2 mg. procaine penicillin G per lb. of food. After a preliminary depletion period of one and 2 weeks, respectively on the vit. B_{12}-deficient diet, the chicks were fed the above supplements for an additional 4 or 8 week period. 2. Thiouracil markedly reduced body weight gains and food intake in both breeds of chicks, and increased the grams of food required for each gram of gain in body weight. Vit. B_{12} and penicillin partially or completely counteracted this growth depression and increased appetite. The larger doses of vit. B_{12} were more effective in these respects than the smaller doses. 3. The large increase in thyroid weight and severe inhibition of comb growth induced by thiouracil were not influenced by the vitamin or antibiotic. 4. In general, these results corroborate previous data on the effects of vit. B_{12} and antibiotics on rats fed thiouracil or following parathyroidectomy"

Michigan State College
East Lansing, Mich.

Rats

645 STERN J R., and McGINNIS J *Antibiotics and early growth of rats fed a soybean oil meal diet*, Arch. Biochem. 28 364-370 Oct. 1950.

The diet used in this study contained soybean oil meal as protein and glucose as carbohydrate. Growth with B_{12} plus antibiotics aureomycin, terramycin or streptomycin was better than with vitamin B_{12} alone.

"It is likely that the increase in growth rate is mediated through alterations in the intestinal flora of the rat, and that the carbohydrate source as well as the antibiotic, is capable of changing the bacterial population of the digestive tract."

646 CRAVIOTO-MUNOZ, J., PONCHER, H. G., and WAISMAN H. A. *Vitamin B, sparing action of aureomycin in the rat*, Proc. Soc. Exper Biol. & Med. 77 18-19 May 1951.

Aureomycin included in the purified diet of rats at levels of 5 and 10 mg per 100 Gm. gives growth increases equal to or better than those obtained with vitamin B_{12} at a level of 0.01 mg. per 100 Gm. This may be explained by the suppression, in the intestine, of bacteria which utilize vitamin B_{12} for their own growth and make it unavailable to the rat, or it may be that the suppression of some microorganisms allows others which synthesize vitamin B_{12} to increase in number

University of Illinois College of Medicine
Chicago, Ill.

647 MEITES, J., and OGLE, R. C. *Antithyrotoxic effects of antibiotics in rats*, Proc. Soc. Exper Biol. & Med. 77 758-761 Aug. 1951.

Authors summary "(1) Three crystalline antibiotics were fed to 100 male and female rats to determine whether they could counteract the retardation of growth induced by feeding thyrotoxic amounts of Protamone. On a ration adequate in vitamin B_{12}, penicillin or neomycin but not streptomycin were completely effective in overcoming the growth-inhibition, while on a ration deficient in vitamin B_{12}, all 3 antibiotics were only partially effective. On the deficient ration, the antibiotics reduced the survival rate of the hyperthyroid rats. (2) At least part, if not all, of the effectiveness of the antibiotics in counteracting growth retardation could be attributed to the increase in food consumption and to an increased efficiency in converting food into body weight gains. The antibiotics did not alter the 74% increase in basal oxygen consumption induced by the Protamone, nor did they change the action of the latter on adrenal and spleen weight. Thymus atrophy was partially or completely prevented by the antibiotics."

Michigan State College
East Lansing, Mich.

648. MEITES J *Counteraction of cortisone inhibition of body hair and thymus growth by vitamin B_{12} and aureomycin*, Proc. Soc. Exper Biol. & Med. 78 692-695 Dec. 1951.

Inhibition of body hair and thymus growth induced in vitamin B_{12}-deficient rats by the subcutaneous injection of 0.5 mg. of cortisone daily was completely or partially prevented by incorporating 200 mcg. of vitamin B_{12} per kg. of ration or 0.005 per cent of aureomycin. Vitamin B_{12} was more effective than aureomycin, and the combination of both was more effective than either alone. Increase in food consumption and greater efficiency in converting food into gains in body weight was present during vitamin and antiobiotic treatment. The author concludes that large doses of cortisone aggravate existing vitamin B_{12} deficiency and that this accounts, at least partially for inhibition of body and hair growth. Experiments are under way which indicate that large doses of cortisone (1 mg. daily) may induce deficiencies of vitamin B_{12} even when a diet adequate in this vitamin is given. The action of vitamin B_{12} and aureomycin upon the thymus appears to be direct, but its mechanism is still unknown.

An addendum mentions the appearance, while this paper was in press, of an article by Wahlstrom and Johnson (*Proc. Soc. Exper Biol. & Med.* 78 112, 1951 [Abstr 655]) which appears to confirm the theory that large doses of cortisone increase requirements for vitamin B_{12}.

Michigan State College
East Lansing, Mich.

649 MEITES, J *Beneficial effects of vitamin B_{12} and aureomycin in rats given large doses of cortisone,* Proc. Soc. Exper Biol. & Med. 81 307-311 Oct. 1952.

Author s summary: "1. Fifty young male albino rats were uniformly divided into 5 equal groups and placed on a purified casein-glucose diet. After a preliminary period of 2 weeks, all except a control group were injected for 28 days with 1 mg. of cortisone acetate daily with or without vitamin B_{12} and/or aureomycin. 2. Cortisone acetate alone induced complete inhibition of body growth, severe alopecia and marked atrophy of the thymus gland. Vitamin B_{12} (200 µg./kilo diet) or aureomycin (0.005%) largely counteracted these inhibitory effects of cortisone acetate, and the combination of the two was more effective than either alone. It was concluded that large doses of cortisone acetate given to young rats on a casein-glucose diet, increased their requirements for vitamin B_{12} and other factors. These data corroborate results previously obtained in a similar experiment on rats fed a corn-soybean meal diet."

Michigan State College
East Lansing, Mich.

Swine

650. CARPENTER, L. E. *Effect of aureomycin on the growth of weaned pigs,* Arch. Biochem. 27 469-471, July 1950.

Pigs fed a diet of natural feedstuffs including animal by-products may respond to additional dietary vitamin B_{12}.

651 SPEER, V C., VOHS, R. L., CATRON D. V., MADDOCK, H. M., and CULBERTSON C. C. *Effect of aureomycin and animal protein factor on healthy pigs,* Arch. Biochem. 29 452-453, Dec. 1950.

The improvement of pig gains by the addition of APF may be explained by the contribution of the APF preparations used of additional vitamin B_{12} and/or their content of unidentified growth factors.

652. CUNHA, T J., MEADOWS G B., EDWARDS H. M., SEWELL, R. F., PEARSON A. M., and GLASSCOCK, R. S. *A comparison of aureomycin, streptomycin, penicillin and aureomycin-B_{12} feed supplement for the pig* Arch. Biochem. 30 269-271 Feb. 1951.

A feed supplement containing penicillin and vitamin B_{12} had no beneficial effect on the growth of pigs. A supplement containing vitamin B_{12} and streptomycin stimulated growth to a small extent. Supplementation of the diet with vitamin B_{12} and aureomycin produced a considerable increase in growth of the pigs.

Florida Agricultural Experiment Station
Gainesville Fla.

653 CARPENTER, L. E. *The effect of antibiotics and vitamin B_{12} on the growth of swine* Arch. Biochem. & Biophysics 32 187 191, June 1951.

Aureomycin, terramycin, penicillin, streptomycin, and chloromycetin stimulated the growth of pigs at the level tested, under the environmental conditions imposed. All the antibiotics except chloromycetin controlled diarrhea. Vitamin B_{12} and streptomycin given alone, either orally or parenterally stimulated the growth of weaned pigs. No greater effects were obtained when both were given together The vitamin had no effect on the incidence of diarrhea, but streptomycin administered either orally or parenterally controlled diarrhea.

654 CATRON D V., MADDOCK, H. M., SPEER, V C., and VOHS, R. L. *Effect of different levels of aureomycin with and without vitamin B_{12} on growth fattening swine,* Antibiotics & Chemother 1 31-40, April 1951

Aureomycin hydrochloride significantly increased weight gain and feed efficiency Crystalline vitamin B_{12} increased weight gain but not feed efficiency There was no complementary effect of aureomycin and vitamin B_{12}.

655 WAHLSTROM, R. C., and JOHNSON R. C. *Effect of cortisone and of aureomycin on baby pigs fed a vitamin B_{12} deficient diet,* Proc. Soc. Exper Biol. & Med. 78 112-114, Oct. 1951

Aureomycin did not alleviate and cortisone enhanced the experimental vitamin B_{12} deficiency in baby pigs.

Dogs

656. ARNRICH, L., LEWIS, E. M., and MORGAN A. F : *Growth of dogs on purified diet plus aureomycin and/or Vit. B_{12},* Proc. Soc. Exper Biol. & Med. 80 401-404, July 1952.

Authors summary: "Eleven young cocker spaniels from 2 litters were fed from weaning for 20 weeks a purified complete diet which had been shown previously produce optimum growth. Vit. B_{12} at the level of 5 ı aureomycin hydrochloride at the level of 10

both these substances were added to the diet of some of the animals. No differences resulted in nitrogen retention or in the nitrogenous constituents of the blood of any of the dogs. Two of the 3 dogs which received vit. B_{12}, 3 of the 4 which received both vit. B_{12} and aureomycin and one of the 2 which received only aureomycin gained more weight per 100 g. food than did the 2 controls and the 3 others. These overgrown dogs had greater total gains, greater empty carcass weights, lower body specific gravity more carcass fat, and more fat-rich adipose tissue than the 5 others. However the fat free carcasses were not significantly heavier It is concluded that better food utilization may result in dogs in some cases from the addition of vit. B_{12} and/or aureomycin to a complete purified diet and that this growth is due solely to increased fat deposition."

University of California
Berkeley, Calif.

METABOLISM

Amino Acids; Vitamins; Related Components

Chicks

657 RICHARDSON, L. R., BLAYLOCK, L. G., GERMAN, H. L., and SHERWOOD R. M. *Amino acids and vitamin B_{12} as supplements to plant proteins for growing chicks*, Federation Proc. 8 393, March 1949

Chicks on a cottonseed meal diet supplemented with lysine weighed more than those on the diet without the amino acid. Tryptophane and methionine increased the weight still more.

A vitamin B_{12} concentrate added to a cerelose-cottonseed meal diet supplemented with lysine, tryptophane and methionine increased the weight of chicks. Three chicks on the diet without vitamin B_{12} weighed, on the average, 228 Gm., while 3 on the diet with 40 mcg. vitamin B_{12} per Kg. averaged 300 Gm. Two groups of 17 chicks each on the cerelose-soybean meal diet averaged 250 Gm. without and 285 Gm. with vitamin B_{12}.

658. SCOTT M. L., HILL, C. H., and NORRIS, L. C. *Factors concerned in the liberation of pteroylglutamic (folic) acid from its heptaglutamate by chick liver* Federation Proc. 8 249 March 1949

Experiments are reported which indicate that vitamin B_{12} is required in addition to p-pyracin in the enzymatic release of folic acid from pteroylheptaglutamate. Both vitamin B_{12} concentrates and crystalline vitamin B_{12} have been added to the liver incubation system with and without p-pyracin, in these experiments.

659 GILLIS M. B., and NORRIS, L. C.: *Effect of the animal protein factor on the requirement for methylating compounds*, J Biol. Chem. 179 487-488, May 1949 (Letter to Editors)

In experiments with chicks, a diet complete in all known vitamins and composed of mixed cereals, plant protein, and supplementary minerals and vitamins was significantly improved by additions of betaine or choline. Since betaine was as effective as choline in promoting the growth of chicks on this diet, the effect was probably due to the increased methyl groups provided. However the addition of 0.15 per cent of liver extract paste was considerably more effective than betaine or choline in promoting growth. No further improvement was obtained by adding betaine or choline to the diet when liver paste was included. The amount of choline contributed by the liver paste was negligible. The liver paste used is a source of animal protein factor (APF) and contains approximately 17 micrograms of vitamin B_{12} activity per gram by assay with Lactobacillus leichmannii. The results obtained in these studies show that the inclusion of a source of APF relieved the need for supplementary methylating compounds, indicating that at least one metabolic function of APF is concerned with transmethylation. It is probable either that transmethylation is more efficient in the presence of an adequate amount of APF or that a partial deficiency of APF creates or stimulates metabolic processes which require excess methyl groups. Further work is required to show whether the activity of the liver paste is due to vitamin B_{12} or to some other component of APF

Cornell University
Ithaca, N Y

660. SCHAEFER, A. E., SALMON, W D., and STRENGTH, D R.: *Interrelationship of vitamin B_{12} and choline. II Effect on growth of the chick*, Proc. Soc. Exper Biol. & Med. 71 202-204, June 1949

The choline requirement of chicks on a choline-low basal diet supplemented with choline was significantly reduced by giving supplements of vitamin B_{12}. Crystalline vitamin B_{12} fed at a level of 15 mcg. per Kg. of diet was as effective as vitamin B_{12} concentrate in supplementing a diet at a choline level of 0.1 per cent over a two-week feeding period. These findings indicate that dietary choline has a significant sparing action on vitamin B_{12}.

661 WILLIAMS, J N., NICHOL, C. A., and ELVEHJEM, C. A. *Relation of dietary folic acid and vitamin B_{12} to enzyme activity in the chick*, J Biol. Chem. 180 689-694, Sept. 1949

Chicks fed a diet supplemented with vitamin B_{12} without folic acid show higher D-amino acid oxidase activity than those not receiving vitamin B_{12}.

662. CHARKEY L. W., WILGUS, H. S., PATTON A. R., and GASSNER, F X. *Vitamin B-12 in amino acid metabolism*, Proc. Soc. Exper Biol. & Med. 73 21 24, Jan. 1950.

In chicks on a basal diet supplemented with the equivalent of 50 mcg. of vitamin B_{12} per Kg. of feed a reduction occurred, as compared with control chicks, in the blood levels of nonprotein nitrogen and of seven individual amino acids (arginine, lysine, methionine, tryptophane, histidine, threonine, valine) The chicks given vitamin B_{12} grew more rapidly and utilized feed more efficiently than the B_{12}-deficient controls, although the

latter had higher blood levels of amino acids. Vitamin B_{12} appears to function in metabolism by enhancing utilization of circulating amino acids for building fixed tissues.

Colorado Agricultural Experiment Station
Fort Collins, Colo.

663. JUKES, T H., STOKSTAD E. L. R., and BROQUIST H. P: *Effect of vitamin B_{12} on the response to homocystine in chicks*, Arch. Biochem. 25: 453, Feb. 1950 (in Letters to the Editors)

"Homocystine, with or without betaine, did not promote the growth of vitamin-B_{12}–deficient chicks on a diet deficient in methionine. However these chicks responded to the addition of methionine. When supplemented with B_{12}, the chicks responded to methionine, homocystine, or homocystine *plus* betaine.

" Vitamin B_{12} may be needed for the transformation of homocystine to methionine in chicks. Other functions for vitamin B_{12} in the nutrition of chicks are indicated by the observation that growth was more rapid on methionine *plus* B_{12} than on methionine alone, and by the finding that vitamin B_{12} is needed for growth and survival of chicks on a diet of natural foods supplemented with methionine and choline."

Lederle Laboratories
Pearl River N Y

664 MACHLIN, L. J., MILLIGAN, J L., DENTON C. A., and BIRD H. R.: *Effect of various amino acids and vitamin B_{12} on chick growth*, Federation Proc. 9 364 March 1950.

665. GILLIS M. B., and NORRIS L. C. *The effect of vitamin B_{12} on the response of chicks to betaine and choline*, J Nutrition 43: 295-302, Feb. 1951

Authors summary "Under the experimental conditions reported above the addition of betaine or choline to a practical ration based on cereals and vegetable protein resulted in a significant improvement of the ration as measured by chick growth. In several experiments with White Leghorn chicks having normal body stores of vitamin B_{12}, the growth responses due to either betaine or choline supplements were approximately equal. Chicks having subnormal reserves of vitamin B_{12}, however utilized betaine more effectively than choline. In 5 experiments with vitamin B_{12}-depleted chicks a significantly greater gain in weight resulted from adding betaine to the basal diet than from adding choline. The evidence suggests that at least one mechanism by which the chick utilized choline is impaired by a deficiency of vitamin B_{12}, while betaine retains its effectiveness under the same conditions."

Cornell University
Ithaca, N Y

666. JUKES, T H., and STOKSTAD E. L. R.: *Studies of vitamin B_{12}, choline and related factors in the diets of chicks* J Nutrition 43 459-467 April 1951.

Authors *summary* 'Choline deficiency as produced by a purified diet was studied in chicks which were also deficient in vitamin B_{12}. The requirement of choline for maximum growth was found to be greater in the absence of vitamin B_{12} than in its presence. However the amount of choline required for the prevention of perosis was not decreased by supplying vitamin B_{12}. Betaine did not have a marked effect when added to diets containing an insufficient level of choline. A methionine-deficient diet was fed to vitamin B_{12}-deficient chicks and no evidence of a sparing effect of vitamin B_{12} on the methionine requirement was noted under the experimental conditions encountered. Choline deficiency was not encountered on a diet of natural foods containing a high level of soybean meal."

Lederle Laboratories
Pearl River N Y

667 GILLIS, M. B., and NORRIS L. C.: *Methylation of homocystine by chicks deficient in vitamin B_{12}* Proc. Soc. Exper Biol. & Med. 77 13-15, May 1951.

Chicks severely depleted of their vitamin B_{12} reserves retain the ability to perform the overall reaction homocystine plus labile methyl forms methionine. If vitamin B_{12} is needed for the completion of this reaction, the amount required must be exceedingly small. It seems probable, however that vitamin B_{12} is concerned in the synthesis of methyl groups or the utilization of such synthesized groups by the chick.

Cornell University
Ithaca, N Y

668. SCHAEFER, A. E., SALMON W D., and STRENGTH, D R. *The influence of vitamin B_{12} on the utilization of choline precursors by the chick*, J Nutrition 44 305-311, June 1951.

Data are presented which indicate that vitamin B_{12} is involved in the utilization of choline precursors for choline synthesis.

669 JUKES, T H.: *Factors involved in utilization of homocystine by chicks* Federation Proc. 11: 447 March 1952.

"Vitamin B_{12}-depleted chicks were fed diets deficient in methionine, choline and vitamin B_{12}. The response to homocystine was markedly increased by vitamin B_{12} under various dietary conditions. However in the absence of vitamin B_{12} homocystine often depressed growth. The presence or absence of vitamin B_{12} or folic acid had little or no effect on the response to methionine. Choline appeared to be highly effective as a methylating agent for homocystine in the presence of vitamin B_{12} for chicks on a diet markedly deficient in methionine. The response to vitamin B_{12} was greater in the presence of folic acid than in its absence and was greater in the presence of homocystine than in its absence when methionine was omitted. The response to folic acid was usually diminished by adding choline. The addition of homocystine and vitamin B_{12}, or homocystine and choline to the basal diet gave poor growth, but excellent growth was obtained when homocystine, vitamin B_{12} and choline were all three added. With vitamin B_{12} added, betaine was much less effective than choline in improving the response to homocystine unless dimethylaminoethanol was added, in whi case betaine was almost as effective as choline. S formate or additional glycine had little effect

utilization of homocystine for growth in the presence of vitamin B_{12}. No response to methionine was obtained in chicks deficient in both pyridoxine and methionine. The requirement for pantothenate was not significantly affected by vitamin B_{12}-deficiency in chicks on a diet adequate in methionine and choline."

Lederle Laboratories
Pearl River N Y

670. SCHWEIGERT B. S. *Amino acids and vitamins*, Chem. & Engin. News 30 1289 March 31, 1952 (in Soc. Proc.)

"Reaction mechanisms involved in the conversion of tryptophan to niacin were outlined. The production of quinolinic acid from the intermediate compound, 3-hydroxyanthranilic acid, is unique in that this involves the conversion of a benzene-type compound to a pyridine-type compound. "

The speaker "suggested that riboflavin and vitamin B_6 function in a catalytic manner In response to a question he indicated that a growth rate reduced because of high levels of glycine might be restored by the addition of vitamin B_{12} to the diet. [He] pointed out that the presence of B_{12} has been shown to reduce the choline requirement necessary for chick growth.

"The conversion of tryptophan to niacin is the best example of an amino acid as a direct precursor to a vitamin. "

American Meat Institute Foundation
Chicago, Ill.

Rats

671 SCHAEFER, A. E., SALMON, W D., and STRENGTH, D R. *Relation of vitamin B_{12} to choline requirement of the rat and chick*, Federation Proc. 8 395, March 1949

The severity and incidence of renal hemorrhage in weanling rats on diets deficient in choline and substitute nutrients was decreased by supplementing the diet with a vitamin B_{12} concentrate. The incidence of renal hemorrhage within 14 days in chicks on a diet supplemented with 0.04% of choline was 100% and on a diet supplemented with 0.06% was 66% The addition of a concentrate supplying 3 mcg of vitamin B_{12} per 100 Gm. of diet to diets supplemented with 0.03, 0.04, and 0.06% of choline reduced the incidence of renal damage to 50% 36% and 0, respectively Preliminary results indicated that on the basis of methyl equivalents DL-methionine had 100% of the theoretical potency of choline chloride when vitamin B_{12} was added to the diet. A diet which required a supplement of 0.6% of choline to induce maximum growth in the absence of vitamin B_{12} resulted in an increased growth increment of 75 Gm. in six weeks when supplemented with 3 mcg. of vitamin B_{12} per 100 Gm. At eight weeks the weight gain of chicks receiving only 0.2% of choline plus vitamin B_{12} was equal to that of chicks receiving 0.6% of choline without vitamin B_{12}.

672. SCHAEFER, A. E., SALMON W D., and STRENGTH, D R. *Interrelationship of vitamin B_{12} and choline. I Effect on hemorrhagic kidney syndrome in the rat*, Proc. Soc. Exper Biol. & Med. 71 193-196, June 1949

Incidence and severity of renal injury in weanling rats fed diets low in choline and methionine were significantly decreased by supplementing the diet with a vitamin B_{12} concentrate or crystalline vitamin B_{12}. Under the conditions of these experiments, 30 mcg vitamin B_{12} per Kg. of diet could replace about half of the supplementary choline or methionine required for protection against kidney damage. When rats on subprotective levels of choline were given vitamin B_{12} in addition, their weight increased significantly but the addition of vitamin B_{12} to an adequate level of choline did not produce an increase in weight gain. These findings establish the existence of an interrelationship between vitamin B_{12} and choline or methionine.

Alabama Polytechnic Institute
Auburn, Ala.

673 SCHULTZE, M. O *Nutritional value of plant materials. II Prevention of acute uremia of the newborn rat by vitamin B_{12}*, Proc. Soc. Exper Biol. & Med. 72 613-616, Dec. 1949

Author's summary: "Acute uremia of the newborn rat observed on rations in which a commercial soybean protein and DL-methionine furnish the only source of amino acids can be prevented by subcutaneous administration of 0.05 μg. of vitamin B_{12} shortly after birth."

University of Minnesota
St Paul, Minn.

674 SCHAEFER, A. E., SALMON W D., STRENGTH, D R., and COPELAND D H. *Interrelationship of folacin, vitamin B_{12}, and choline. Effect of hemorrhagic kidney syndrome in the rat and on growth of the chick*, J Nutrition 40 95-112, Jan. 1950 (abstr J Am. Dietet. A. 26 450, June 1950)

"The incidence and severity of renal injury in weanling rats fed diets low in choline and methionine were decreased by supplementing the diet with folacin. When both folacin and vitamin B_{12} were added to the basal diet supplemented with 0.04 per cent choline chloride or 0.128 per cent dl-methionine, there was complete protection against kidney damage. Evidence is offered that the nutritional requirements of the chick and rat for folacin, vitamin B_{12}, and choline are interrelated, and a specific requirement for one of these nutrients cannot be established unless the level of the other two is taken into consideration."

675 STEKOL, J A., BENNETT M. A., WEISS, K., HALPERN P., and WEISS, S.: *Sulfur-containing amino acids in growth with a 'labile methyl' free diet containing vitamin B_{12}*, Federation Proc. 9 234 March 1950.

"We reported previously that 30 days or older rats are able to grow on a diet which was free of all the known 'labile methyl group donors but which contained vitamin B_{12} and homocystine (*Abstracts, Am. Chem. Soc.*, 116th meeting, Sept. 18, 1949 55C) The amino acid diet which was used in the above studies was complete with respect to all the essential amino acids, except for methionine, and it contained all the known vitamins, including folic acid and biotin. We now find that on this diet, in the presence of vitamin B_{12}, homocysteine produces even better growth response than equivalent amounts of homo-

cystine. Substitution of homocysteine by L- and L-*allo*-cystathionine resulted in cessation of growth, which was resumed as soon as the cystathionines were replaced by homocystine. On a diet containing ample methionine as the sole sulfur-amino acid, but which was free of vitamin B_{12}, poor growth was obtained supplementing the methionine-containing diet with vitamin B_{12} resulted in good growth. None of the experimental animals receiving vitamin B_{12} died of kidney lesions in spite of the complete absence of "labile methyl group donors in the diet."

Lankenau Hospital Research Institute and Institute for Cancer Research Philadelphia, Pa.

676. DUBNOFF J W *Effect of vitamin B_{12} on the reduction of S-S compounds in vitro* Federation Proc. 9 166, March 1950.

"Addition of vitamin B_{12} concentrates to liver slices and homogenates increases the reduction of S-S compounds by preformed and added hydrogen donors. The specificity of hydrogen donors and the relation of this effect to methionine synthesis from homocystine will be discussed."

California Institute of Technology Pasadena, Calif.

677 SALMON W D: *Effects of protein level, vitamin B_{12}, and folacin on utilization of methionine for transmethylation*, Federation Proc. 9 369 March 1950.

"Weanling rats were fed choline-free diets containing 7 or 9% of casein and corn gluten meal and sufficient DL methionine to prevent kidney lesions in a 2-week period. When 10% of gelatine was added, at the expense of sucrose no kidney damage occurred the further addition of 0.10% DL-tryptophane resulted in 100% incidence of renal damage but tryptophane without gelatine had no effect. Vitamin B_{12} alone did not decrease incidence of kidney damage by the gelatine-tryptophane-containing diet but vitamin B_{12} and folacin together decreased incidence 50% A 50-100% incidence of renal injury developed in rats receiving a choline-free, synthetic diet containing 60% of alcohol extracted casein. Vitamin B_{12} and folacin, or these vitamins together with 0.64% DL methionine, did not consistently increase growth or decrease incidence of renal damage. Choline alone (0.20%) prevented kidney lesions but had little effect on growth. Choline together with vitamin B_{12} and folacin produced normal growth and grossly normal kidney condition; ethanolamine appeared to be as effective as choline in this combination. The results indicate a significant effect of protein level on utilization of methionine and a specific relationship of vitamin B_{12} and folacin to transmethylation in the rat."

678. SIMMONDS, S., KELLER, E. B., CHANDLER, J P., and DU VIGNEAUD V *The effect of ethionine on transmethylation from methionine to choline and creatine in vivo* J Biol. Chem. 183 191 195, March 1950

Experiments with rats showed that ethionine decreased the amount of transmethylation from methionine to choline by about 20 per cent.

Cornell University Medical College New York, N Y

679 SCHULTZE, M O *Nutritional value of plant materials. I Growth of rats on purified rations containing soybean protein*, J Nutrition 41 103-113, May 1950.

Author s summary "Rats fed purified rations containing 24% protein in the form of a soybean protein preparation and DL-methionine as the only source of amino acids made satisfactory post weaning weight gains for 4 successive generations. Extensive purification of the soybean protein did not decrease the growth rate of rats consuming this protein as compared to that of animals consuming a less purified preparation. Daily administration of 0.25 µg. of crystalline vitamin B_{12} to rats on a ration containing a highly purified soybean protein preparation did not increase the early post-weaning weight gains or the efficiency of food utilization."

University of Minnesota St. Paul, Minn.

680. DUBNOFF J W *The effect of B_{12} concentrates on the reduction of S-S groups*, Arch. Biochem. 27 466-467 July 1950.

The influence of vitamin B_{12} on transmethylation reactions was studied. The results suggest a B_{12} component acting as a hydrogen carrier to -S-S- groups. This may explain the effect of B_{12} on methionine formation and the fact that B_{12} may be at least partially replaced as a microbiological growth factor by reducing agents or by lowering the oxygen tension of the medium. Other apparently diverse in vivo activities of B_{12} which have been reported may be the result of the influence of B_{12} on glutathione and S-H groups which in turn can activate a wide variety of enzyme systems.

681 DINNING J S., PAYNE, L. D., and DAY P L. *The requirements of rats for methyl groups and vitamin B_{12} in the production of leucocytes* Arch. Biochem. 27 467-469 July 1950 (Letter to Editors)

Results of a study of the requirement of labile methyl for leukocyte formation and of the effect of vitamin B_{12} in the utilization of methyl groups suggest that B_{12} may be essential for the utilization of methyl groups of betaine.

682. WEISSBACH, A., ELWYN D., and SPRINSON D B. *The synthesis of the methyl groups and ethanolamine moiety of choline from serine and glycine in the rat*, J Am. Chem. Soc. 72 3316-3317 July 1950 (in Communications to the Editor)

Experiments are reported which show that the rat can synthesize methyl groups from serine, or from glycine converted to serine. This is in agreement with the recent report (Bennett, *Science* 110: 589 1949) that diets devoid of methyl group donors will support growth when folic acid and vitamin B_{12} are present.

Columbia University College of Physicians and Surgeons New York, N Y

683 CARTER, R. E., BUSCH, E., and STRANG, V *The effect of vitamin B , on the leukopenia induced by radiation*, Blood 5 753-757 Aug. 1950.

Observation that thymidine can replace B_{12} as a growth factor for lactobacillus had led to the postulation that B_{12} functions as a coenzyme in the conversion of thymine to thymidine. To determine a possible interference with nucleoprotein formation produced by radiation, on the basis of its known effects on living tissue, a trial of the effects of vitamin B_{12} on radiation-induced leukopenia in rats was made.

Authors conclusions "Crystalline vitamin B_{12} administered via intramuscular injection, in single and in multiple doses, produced no effect on the leukopenia induced in rats by 400 roentgens of 250 kv x-rays."

Los Alamos Scientific Laboratory
University of California
Los Alamos, N M.

684 DuVIGNEAUD V., RESSLER, C., and RACHELE, J R. *The biological synthesis of "labile methyl groups,"* Science 112 267 271, Sept. 8, 1950.

This paper is a résumé of the present status of the concept of the synthesis of biologically labile methyl groups. The authors first review a number of studies made by other workers on the growth of rats maintained on a methionine-free, homocystine-containing diet supplemented with choline, with crude liver extract, or with vitamin B_{12} alone or together with folic acid. It appeared likely that vitamin B_{12} and folic acid may be involved in the synthesis of labile methyl groups.

The authors then report the results of investigations with germ free rats (conducted in collaboration with James A. Reyniers, Thomas D Luckey *et al.* at LOBUND of the University of Notre Dame) the purpose of which was to obtain evidence as to where the labile methyl groups are synthesized in the tissues, or in the intestines, mediated through bacterial action. The technic used involved the detection of the synthesis of methyl groups through the isolation of choline from rats, the body water of which contained deuterium oxide (D_2O) Rats of the LOBUND strain were maintained with D_2O in their drinking water under both germ-free and nonsterile conditions at LOBUND. For purposes of comparison, animals of the Rockland Farms strain were kept at the Cornell Laboratories in New York under ordinary laboratory conditions on the same dietary regimen as that used at LOBUND. All determinations were made in New York, the LOBUND sterile specimens being shipped there by air express. All animals received the LOBUND diet (identified in detail) one pair of rats received additional supplements of vitamin B_{12}. It was found that deuterium was present in significant amounts in the methyl groups of choline isolated from the germ-free animals; this means that these animals were able to synthesize methyl groups. In the LOBUND animals maintained under nonsterile conditions a higher value (which may not be significant) was obtained. The authors believe that, in these animals, additional synthesis of labile methyl groups may have been mediated through intestinal bacteria. The diets enriched with additional vitamin B_{12} did not increase the degree of methyl synthesis. It is concluded that biologically labile methyl groups can be synthesized by the tissues of the rat.

The authors then interpret the results of other workers in the light of their findings. They believe that, although labile methyl groups can be synthesized by the animal, the rate of synthesis is not fast enough for the demands of the growing young rat. Biologically labile methyl groups can, therefore, be regarded as an essential dietary component.

Thirty-three references are cited.

Cornell University Medical College
New York, N Y

685. STEKOL, J A., and WEISS K.: *Vitamin B_{12} and growth of rats on diets free of methionine and choline,* J Biol. Chem. 186 343-350, Sept. 1950.

Rats aged 30 days or more maintained on diets free of labile methyl groups and containing homocystine but no vitamin B_{12} continued growing for two or three weeks and then lost weight and died with severely hemorrhagic kidneys. If vitamin B_{12} was administered during the period of loss of weight the animals immediately resumed growth. All rats continued growing on such diets if vitamin B_{12} was included from the start. The initial growth of rats on vitamin B_{12}-free diets is probably a function of the B_{12} stored in the liver Replacing a portion of homocystine by cystine, or replacing homocystine by homocysteine resulted in better growth rates. Replacing the homocystine by a mixture of L-cystathionine and L-allocystathionine resulted in loss of weight in spite of the presence of vitamin B_{12}. If choline was added to the cystathionine diet, slow growth was obtained.

Rats aged 22 to 24 days did not as a rule survive on the labile methyl free diet in spite of the presence of vitamin B_{12}, and usually developed damaged kidneys.

The authors conclude that the rat is able to synthesize methionine and, probably other metabolites containing the labile methyl group and that vitamin B_{12} and other dietary factors may play a decisive role in the synthesis and utilization of the synthesized labile methyl groups. The possible role of amino acids, particularly glycine and serine, in the synthesis is pointed out.

Lankenau Hospital Research Institute, and
The Institute for Cancer Research
Philadelphia, Pa.

686. VIVANCO F., JIMENEZ DIAZ, C., and PALACIOS, J *Recent studies on cicerism and nature of CH factor* Rev Clin. Espan. 39: 168- Nov 15, 1950 (abstr J.A.M.A. 146 218, May 12, 1951)

Rats were given a diet in which the only source of protein was the albumin of the chick pea. The rats were divided into five groups, depending upon the dietary supplements given. The first group served as controls, the second received methionine, the third received vitamin B_{12}, the fourth received crude liver extract, and the fifth was given steamed liver extract. All of the animals receiving only the basal diet developed cicerism (the equivalent of lathyrism in humans) Cicerism did not develop in the animals that received methionine or the liver extracts. Cicerism occurred in 87.5 per cent of the animals that received vitamin B_{12} supplements. The experiments show the existence of a vitamin, which the authors call the CH factor in liver extracts. This vitamin is water soluble and thermoresistant. Apparently the CH factor is necessary in the absence of a certain level of choline or methionine in the diet. The authors believe that the CH factor intervenes in the metabolic processes in such a way as to

correct deficiencies of methionine and choline. The CH factor is considered as being one of the essential components of the animal protein factor Methionine can be substituted for the CH factor

687 SAKAMI, W., and WELCH, A. D *Synthesis of labile methyl groups by the rat in vivo and in vitro* J Biol. Chem. 187: 379-484 Nov 1950.

688. BENNETT M. A.: *Utilization of homocystine for growth in presence of vitamin B_{12} and folic acid* J Biol. Chem. 187 751 756, Dec. 1950

Rats fed a "labile methyl" free diet containing 0.83 per cent homocystine as the sole source of sulfur amino acids and the eight B vitamins gradually stopped growing if their intestinal synthesis of folic acid was suppressed by sulfasuxidine. When this leveling had occurred, adding folic acid to the diet caused resumption of growth in some instances, but not in all, and growth eventually stopped even in the animals that had resumed growth. This seemed to indicate that an additional factor was involved. Addition of crystalline vitamin B_{12} to the vitamin supplement at this point caused a resumption of growth in all animals. After vitamin B_{12} was discontinued, the animals continued to grow for a short period, after which the curves slowly leveled. Substitution of methionine for homocystine at the point mentioned above caused a resumption of growth without the addition of B_{12}. The proprietary liver extract used gave growth proportional to its vitamin B_{12} content, on addition of folic acid. The previous negative results obtained with a proprietary concentrated solution of liver extract were evidently due to folic acid deficiency

Lankenau Hospital Research Institute and Institute for Cancer Research Philadelphia, Pa

689 LIENER, I. E., and SCHULTZE, M. O *Liver arginase activity as related to blood urea in acute uremia of new-born rats* J Biol. Chem. 187 743-750, Dec. 1950.

Authors summary "The acute uremia observed in new-born rats from mothers maintained on rations containing crude plant materials was accompanied by a significant increase in the activity of liver arginase, compared to rats of normal appearance of the same age in which the maternal diet had been supplemented with fish solubles.

"Liver arginase activity closely paralleled the marked rise in the concentration of urea in the blood which occurred 12 to 24 hours after birth.

"The early postnatal administration of vitamin B_{12} prevented the abnormal rise in arginase activity and prevented acute uremia in the new-born rat. Under these conditions a higher degree of correlation was found to exist between the arginase activity of the liver and the concentration of urea in the blood."

University of Minnesota St. Paul, Minn.

690. ARNSTEIN H. R. V., and NEUBERGER, A.: *The effect of vitamin B , on the conversion of glycine to choline* Biochem. J 48 iii, Jan. 1951 (in Proc. Biochem. Soc.)

691 DUBNOFF J: *Vitamin B_{12} in methionine formation*, Federation Proc. 10 178, March 1951

"It was previously reported (*Federation Proc.* 9 166, 1950 [Abstr 676] and *Arch. Biochem.* 27 466, 1950 [Abstr 680]) that B_{12} concentrates catalyze the reduction by rat liver slices of homocystine to homocysteine. Further work has shown that crystalline B_{12} in concentrations as low as 0.0001 γ/ml. is also active. Reduced glutathione markedly increases the reduction of homocystine. Independent evidence confirming this role for B_{12} in methionine synthesis based on the replaceability of B_{12} in the B_{12} or methionine requiring mutant of B. Davis by homocysteine will be reported"

California Institute of Technology Pasadena, Calif.

692. SCHAEFER, A. E., KNOWLES, J L., and SALMON W D: *Influence of vitamin B_{12} and folacin on synthesis of choline and methionine by the rat in vivo and in vitro* Federation Proc. 10 393, March 1951.

"Weanling rats fed a choline-low basal diet containing thoroughly methanol-extracted peanut meal and oxidized casein failed to gain in body weight unless the diet was supplemented with DL-methionine. The effectiveness of various precursors for synthesis of choline and methionine *in vivo* were measured by gain in body weight, protection against renal damage and total carcass choline. Replacing the DL-methionine and choline supplements with DL-homocystine and various choline precursors, respectively with and without *vitamin B_{12} and/or folacin* indicated that vitamin B_{12} and folacin were involved in the synthesis of choline and methionine. When the basal diet was supplemented with homocystine and ethanolamine, methylation of these compounds by methyl from betaine occurred only when the diet was supplemented with vitamin B_{12} and folacin. Glycine and serine or methanol and aminoethanol did not prevent the synthesis of sufficient choline for the support of growth and the prevention of fatal renal hemorrhage in weanling rats. *In vitro* studies were made with liver slices from rats receiving the various deficient diets and from chicks receiving similar diets. The addition of vitamin B_{12} and folacin to substrates containing dimethylaminoethanol resulted in a 2 to 4-fold increase in synthesis of free choline."

Alabama Polytechnic Institute Auburn, Ala.

693 GOYCO J A.: *Effect of vitamin B_{12}*, dl-*methionine and vitamin E on the growth promoting value of Torula yeast protein*, Federation Proc. 10 191 March 1951.

"Male Wistar rats 23 days old were used. The basal diet contained 18% protein supplied by *Torula* yeast. Trials lasted 4 weeks. Growth promoting value of *Torula* yeast was 1.23. When this ration was supplemented with 200 μg. B_{12} per kg. the value was 1.40. The difference was found to be not significant. The incidence of hepatic necrosis in both groups was very high. In a group of animals supplemented with the same amount of B_{12} and 2 mg. vitamin E per week given separately the growth promoting value was significant, 1.63. No hepatic necrosis was observed in these rats. In another experiment the same basal ration was used except that it contained 0.5% methionine. One group received this ration only a

group the ration separately supplemented with one µg. B_{12} per day and a third group, the ration separately supplemented with one µg. B_{12} per day and 2 mg. vitamin E per week. Growth promoting values were 2.01, 2.54 and 2.77 respectively. The difference between those on methionine alone and those receiving in addition B_{12} was significant. Not a single one developed hepatic necrosis. B_{12} as well as methionine increased food consumption to about the same extent. When fed together the increase in food intake was almost additive. Methionine, B_{12} and vitamin E together step up the growth promoting value of *Torula* yeast protein to 2.77 a level never observed by us before."

University of Puerto Rico School of Medicine
San Juan, P. R.

694 DINNING, J. S., PAYNE, L. D., and DAY, P. L. *The influence of folic acid, vitamin B_{12}, and methyl donors on white blood cell production in rats.* J. Nutrition 43: 525-531, April 1951.

Authors summary: "Weanling Sprague-Dawley rats were given a basal methionine-deficient, purified diet. Various groups received this diet with supplements of methionine, choline, or betaine, with and without folic acid and vitamin B_{12}. Rats receiving the basal diet alone developed leucopenia. The leucopenia was prevented by supplements of methionine with or without folic acid and vitamin B_{12}, or by choline or betaine with supplements of folic acid and vitamin B_{12}. Supplements of choline or betaine in the absence of added folic acid and vitamin B_{12} failed to prevent leucopenia."

University of Arkansas School of Medicine
Little Rock, Ark.

695 MILLER, E. C., PLESCIA, A. M., MILLER, J. A., and HEIDELBERGER, C. *On the metabolism of 3'-methyl-4-dimethylaminoazobenzene-N-methyl-C^{14} in the rat,* Cancer Research 11: 268-269, April 1951.

"Approximately 70 per cent of the C^{14} from 3'-methyl-4-dimethylaminoazobenzene-N-methyl-C^{14} was expired as carbon dioxide within 48 hours after the administration of a single dose to rats by stomach tube. Approximately 10 per cent of the activity was excreted in the urine and feces, and the remainder was found in the carcass. Of the radioactivity left in the animal after one or several doses, 15-20 per cent was contained in the protein and 5 per cent in the choline, and the specific activity of both of these components was 2-3 times higher in the liver than in the rest of the body. Fifteen to 35 per cent of the activity in the protein was contained in the serine, and all the radioactivity in this amino acid was in the β-position. Since other investigators have shown that formate and formaldehyde are incorporated only into the β-position of serine, at least one N-methyl group from the dye is apparently oxidized through these intermediates or a related one-carbon compound. Most of the radioactivity in the choline was contained in the N-methyl groups, and, although some direct transmethylation cannot be excluded, it seems most likely that the activity was derived from formate or formaldehyde. Rats deficient in either folic acid or vitamin B_{12} incorporated much less radioactivity into the liver serine and choline than control rats supplemented with both of these vitamins."

University of Wisconsin
Madison, Wis.

696. SCHAEFER, A. E., and KNOWLES, J. L. *Influence of vitamin B_{12} and folacin on the synthesis of choline and methionine by the rat,* Proc. Soc. Exper. Biol. & Med. 77: 655-659, Aug. 1951.

Authors summary: "The role of vit. B_{12} and folacin in the biosynthesis of choline and methionine has been studied and the following conclusions are warranted: 1. In the presence of adequate methyls (from choline) vit. B_{12} alone was as effective for methionine synthesis as when combined with folacin. When fed alone, folacin was ineffective. 2. In the presence of a limited supply of methyl groups, both folacin and vit. B_{12} were essential for the normal development of weanling rats. 3. Vit. B_{12} and folacin were essential for the synthesis of choline and of methionine from aminoethanol, homocystine, and a limited supply of betaine. 4. As a methyl donor for the synthesis of methionine from homocystine, betaine was at least as effective as choline, mole per mole. 5. Under the experimental conditions employed, the rate of synthesis of the methyl group or utilization of methanol for the methlyation of homocystine to methionine and aminoethanol to choline was not fast enough to support normal development of weanling rats."

Alabama Polytechnic Institute
Auburn, Ala.

697 HALE, O. M., and SCHAEFER, A. E. *Choline requirement of rats as influenced by age, strain, vitamin B_{12} and folacin,* Proc. Soc. Exper. Biol. & Med. 77: 633-636, Aug. 1951.

Authors summary: "(1) Weanling rats of the Sprague-Dawley (SD) strain have a lower choline requirement for protection against renal hemorrhage and a higher requirement for maintenance of normal liver fat than was observed in rats of the Alabama Experiment Station (AES) strain. (2) Forty-two-day-old rats of either strain grew rapidly and failed to develop hemorrhagic kidneys when they were fed the choline-deficient basal diet supplemented with only vit. B_{12} and folacin. However liver fat of the SD strain rats was 33% (dry basis) as compared to 62% for the AES strain rats. (3) The addition of vit. B_{12} and folacin to the basal diet supplemented with suboptimal choline chloride markedly suppressed the accumulation of fat in the liver, reduced the incidence of renal damage and increased the choline content of the livers of the SD strain of rats. Liver fat values averaging 12.8% (dry basis) were obtained with a 0.20% choline chloride supplement in the presence of vit. B_{12} and folacin as compared to a requirement of 0.40% choline chloride in the absence of vit. B_{12} and folacin."

Alabama Polytechnic Institute
Auburn, Ala.

698. WILLIAMS, J. N.: *Further studies on choline oxidase factors,* J. Biol. Chem. 192: 81-84, Sept. 1951.

Folic acid, Leuconostoc citrovorum factor (LCF), vitamin B_{12}, and ascorbic acid singly and in combination markedly stimulate choline oxidation in liver homogenates of rats fed aminopterin.

The results indicate that folic acid or LCF, vitamin B_{12} and ascorbic acid are important in maintaining choline oxidase.

699 SAHASRABUDHE, M. R., and LAKSHMINARAYAN RAO M. V *Effect of vitamin B_{12} on the synthesis of protein and nucleic acids in the liver* Nature 168 605-606, Oct. 6, 1951

Vitamin B_{12} stimulates both protein and nucleic acid synthesis in the liver

"The greater regenerations of liver protein in animals receiving B_{12} is presumably due to its effect in enhancing amino acid utilization.

"We believe these results are the first experimental proof of the direct intervention of vitamin B_{12} in mammalian nucleic acid metabolism."

700. BENNETT M. A., JORALEMON J., and HALPERN P E. *The effect of vitamin B_{12} on rat growth and fat infiltration of the liver* J Biol. Chem. 193 285-291 Nov 1951

Crystalline vitamin B_{12} or equivalent amounts of this vitamin (as liver or APF concentrate) will enable rats to grow at practically identical rates on a "labile methyl"-free homocysteine diet containing folic acid and to maintain normal livers.

When homocysteine is replaced by methionine in the diet, the rats grow at essentially identical rates either in the absence or presence of crystalline B_{12} or equivalent amounts of other sources of the vitamin.

701. LIENER, I. E., and SCHULTZE, M. O *The excretion of N^1-methylnicotinamide and creatinine as influenced by vitamin B_{12} and various methyl donors*, J Nutrition 46 223-237 Feb. 11, 1952.

Authors summary "Young vitamin B_{12}-deficient rats were fed a labile-methyl free ration with the following supplements in the presence or absence of vitamin B_{12}: none methionine choline homocystine homocystine plus choline, betaine, or formate. The urinary excretion of N^1-methylnicotinamide or creatinine was determined following an intraperitoneal dose of nicotinamide or guanidoacetic acid.

"Only when the basal ration was supplemented with homocystine in the presence of choline, betaine, or vitamin B_{12} were the growth and the excretion of N^1-methylnicotinamide similar to those observed with the methionine-supplemented ration. The administration of vitamin B_{12} effected a further improvement in growth and increase in N^1-methylnicotinamide excretion when the diet contained homocystine plus choline or betaine. Formate in the absence of vitamin B_{12} was ineffective as a methyl donor to homocystine.

"When the basal ration contained homocystine, the administration of vitamin B_{12} or additional supplementation with choline caused an increase in growth and excretion of creatinine comparable to the increase obtained with the methionine-supplemented ration. Choline alone, although incapable of supporting growth, effected a similar increase in creatinine output.

"The implication of these results with respect to the role of the vitamin B_{12} in the biosynthesis of methionine has been discussed."

University of Minnesota
St. Paul, Minn.

702. LANGEMANN H., and KENSLER, C. J *Methionine formation in liver homogenates* Federation Proc. 11 366, March 1952.

"Conditions have been established for the measurement of methionine formation from homocysteine and choline, betaine or dimethylthetin in liver homogenates which yield higher Q methionine values than those previously reported by others. Evidence obtained using succinate to saturate proton and electron acceptors present in tissue homogenates supports Dubnoff's suggestion that in the rat choline must be oxidized before its methyl group is available for methionine synthesis. The use of succinate in the system increased the values obtained when betaine was the methyl donor but greatly reduced the values when choline was the methyl donor The dimethylthetin-homocysteine transmethylase activity of mouse liver was twice that of rat liver Rabbit, guinea pig and chick liver was less active than rat liver chick liver activity being less than ½ that of rat liver Betaine transmethylase activity did not parallel dimethylthetin transmethylase activity among the different species. Rat and mouse liver tumors showed little or no transmethylase activity In chicks deficient in folic acid or Vitamin B_{12}, transmethylase activity was not reduced. The folic acid deficient chicks yielded appreciably higher values than their supplemented controls."

Cornell University Medical College
New York, N Y

703 JOHNSON C. A., KIRCH, E. R., and BERGEIM, O *Effects of methionine ethionine and of certain antibiotics on liver iron*, Federation Proc. 11 236, March 1952.

"Hemachromatosis has been noted in individuals on deficient diets and Kinney Hegsted and Finch (*J Exper Med.* 90 137 1949) observed that methionine added to diets of corn grits led to an increase of liver iron in rats. We have studied the effect on young, growing rats and adult rats on adequate casein containing diets of the addition of 8% methionine over periods of 3-7 weeks. In the younger rats on moderate iron intakes methionine increased liver iron significantly Spleen iron was also increased. Phenylalanine did not have this effect. Ethionine brought about a decrease in liver iron. Vitamin B_{12} led to an increase in spleen but not in liver iron. High doses of neomycin and streptomycin increased liver iron. In adult animals on high iron diets (6% ferric citrate) methionine increased liver and spleen iron. Ethionine alone produced a slight rise in liver iron and a decline in the spleen. Given with methionine it counteracted the effects of methionine on liver and spleen iron. With increases in total iron in the livers the amounts present in HCl extracts as ferrous iron increased. However the ratios of ferrous iron to total iron markedly decreased. Ratios of ferric to ferrous iron in cecal contents showed no clear relation to liver values. Some alterations were noted in acid extractable sulfhydryl compounds of the liver"

University of Illinois
Chicago, Ill.

704 ROSE, I. A., and SCHWEIGERT B. S. *Effect of vitamin B_{12} on nucleic acid metabolism of the rat*, Proc. Soc. Exper Biol. & Med. 79 541-544, March 1952.

This study demonstrates the effect of a vitamin B_{12} deficiency on the amounts of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) on rat organs, and on the rate of incorporation of N_{15} labeled glycine into the purines of RNA. The authors summary reads as follows "1. The amounts of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the livers of rats fed a vit. B_{12} deficient diet were significantly lower per g. of fresh or of dry liver or of liver nitrogen than those for the supplemented rats. No difference was observed, however in the amounts of RNA and DNA per cell for the deficient or supplemented rats. The amount of nucleic acids per g. of spleen or kidney was the same for the deficient and supplemented group. 2. Other studies indicated that the rates of incorporation of N_{15} glycine into the purines of RNA were lower for the deficient animals. 3. These studies suggest that in a vit. B_{12} deficiency the reduced rate of nucleic acid synthesis in the liver reduced the rate of cell divisions."

University of Chicago
Chicago, Ill.

705 STEKOL, J., WEISS S., and WEISS, K.: *Vitamin B_{12} and folic acid in the synthesis of choline in the rat*, Arch. Biochem. & Biophysics 36 5-10, 1952 (abstr Blood 7: 857 Aug. 1952)

"The results are interpreted as indicating the involvement of vitamin B_{12} or of its physiologic derivative in the synthesis of serine from glycine. Folic acid or its physiologic derivative is involved in the synthesis of both moieties of choline from serine, and, through serine, from glycine."

Lankenau Hospital Research Institute, and
Institute for Cancer Research
Philadelphia, Pa.

706. MORGAN A. F., and LEWIS E. M. *Modification of choline deficiency by simultaneous pantothenic acid deficiency* Federation Proc. 11 451 March 1952.

"The effect of choline deficiency upon rats fed normal and pantothenic acid-deficient diets was observed from the 14th to the 56th day of life. In the pantothenic acid–deficient and doubly deficient animals the liver fat remained normal or low and adrenal cholesterol much lower than in the normal and choline-deficient groups even when pair-fed. When vitamin B_{12} was given the liver fats were normal in all cases but adrenal cholesterol remained markedly depressed in the pantothenic acid–deficient rats. Neither the addition of 1% cholesterol to the diet nor the daily injection of adrenocortical extract increased the adrenal cholesterol or the liver fat of animals deprived of pantothenic acid. Blood and serum lipid phosphorus levels were slightly raised in the latter groups. Daily injection of 2 mg. ACTH for 5 days before autopsy produced increases in liver fat of normal animals but none in pantothenic acid–deficient groups. The adrenal cholesterol of ACTH treated animals was somewhat increased but in all the pantothenic acid–deficient rats these levels were less than half that of the normal animals. The thymus weights were greatly reduced in all the pantothenic acid–deficient groups whether ACTH treated or not. Thus, lipid metabolism of pantothenic acid–deficient rats was severely deranged as shown by failure of fat deposition in the liver, loss of adrenal cholesterol, and rise in serum lipid phosphorus. An increase in lipid utilization may occur in this deficiency state."

University of California
Berkeley Calif

707 SCHEPARTZ, B., and NADEL, E. M.: *Effect of ascorbic acid, folic acid and vitamin B_{12} upon tyrosine oxidation in acetone powder preparations of guinea pig liver* Federation Proc. 11 425, March 1952.

"Acetone powder preparations of guinea pig livers when supplemented with α ketoglutarate were found capable of converting l-tyrosine to acetoacetate. As previously reported for powders prepared from rat livers, the catabolism of tyrosine required an initial transamination, with α ketoglutarate as the acceptor of the amino group (B. Schepartz, *J Biol. Chem.* 193: 293, 1951) Enzymatic activity was reduced 50% in livers of scorbutic animals. This deficiency was remedied by the *in vitro* addition of ascorbic acid. Decreased food consumption by scorbutic animals was not the cause of the decline in enzymatic activity for acetone powders prepared from livers of fasted animals that had received oral supplements of ascorbic acid had normal levels of activity *In vitro* additions of folic acid or of vitamin B_{12} did not restore the activity of preparations from scorbutic animals. Aging of the acetone powders prepared from normal livers caused a decrease in the activity of these powders to levels obtained in preparations from scorbutic animals. Normal activity was restored [by] *in vitro* supplementation with ascorbic acid. The data confirm the reports of other laboratories on the importance of ascorbic acid in tyrosine metabolism."

Jefferson Medical College
Philadelphia, Pa.
National Cancer Institute
Bethesda, Md.

708. EDWARDS, C. H., and CARTER, R. T *Interrelationships between vitamin B_{12} and methionine in metabolism*, Federation Proc. 12 413, March 1953.

"Research conducted during the past year suggests that vitamin B_{12} and methionine are intimately related in the metabolism of rats partially depleted of protein reserves. After ad-libitum feeding for 6 days of a diet low in nitrogen but adequate in other nutrients, adult male rats were force-fed a basal ration containing eight essential amino acids (methionine and arginine-free) for a 9-day interval (period I) The animals were divided into groups of 6 and fed daily for 9 days (period II) the basal amino acid diet with either *a*) no supplement, *b*) 25 mg. methionine, *c*) 0.4 micrograms vitamin B_{12}, *d*) 25 mg. methionine and 0.4 micrograms vitamin B_{12}, or *e*) 50 mg. methionine. Nitrogen balances were determined during the last 5 days of periods I and II. Animals receiving a supplement of 25 mg. methionine were in negative nitrogen balance and had high concentrations of liver fat. When the diet was supplemented with vitamin B_{12} and 25 mg. methionine, the animals were in nitrogen equilibrium and the concentrations of liver fat were normal. In the absence of methionine, animals receiving vitamin B_{12} were in negative nitrogen balance. When 50 mg. methionine were fed, nitrogen balance was achieved, but concentrations of liver fat were abnormally high.

Methionine alone, in concentrations which maintained nitrogen balance, stimulated a 50% increase in the production of hemoglobin."

Tuskegee Institute
Tuskegee, Ala.

Swine

709 CUNHA, T J., HOPPER, H. H., BURNSIDE, J E., PEARSON A. M., GLASSCOCK, R. S., and SHEALY A. L.: *Effect of vitamin B_{12} and APF supplement on methionine needs of the pig*, Arch. Biochem. 23 510-512, Oct. 1949 (Letter to Editor)

EXCRETION ABSORPTION RETENTION

710. CHOW B. F., BARROWS, L., and LANG C. *Microbiological activity of vitamin B_{12} in urine of normal rats following oral and subcutaneous administration of this vitamin*, J Nutrition 42 405-414, Nov 1950.

711 CHESTERMAN D C., CUTHBERTSON W F J., and PEGLER, H. F *Vitamin B_{12} excretion studies*, Biochem. J 48 li-lii, April 1951 (in Proc. Biochem. Soc.)

712. BARBEE, K. W., and JOHNSON B C. *Metabolism of radioactive vitamin B_{12} by the rat*, Proc. Soc. Exper Biol. & Med. 76 720-721 April 1951

Radioactive vitamin B_{12} has been given orally or subcutaneously to 4 adult white rats. The radioactivity (entirely B_{12}) was excreted primarily in the urine following injection, while the radioactivity (B_{12} plus inorganic cobalt) was excreted primarily in the feces following oral administration.

713. ABBOTT L. D., JR. *Biochemical changes induced by vitamin B_{12}*, Federation Proc. 10 153, March 1951.

"Nitrogen and phosphorus balances before and after injection of vitamin B_{12} in rabbits on both unrestricted and constant dietary intake have been studied. In rabbits on unlimited intake of Rabbit Chow Checkers, injection of B_{12} (5 µg./day for 5 or 6 days) was accompanied by marked positive nitrogen balance. The animals ate more and retained more nitrogen than in preceding control periods. Phosphorus retention also occurred. The ratio of P:N retained under the influence of B_{12} approximated Benedict's P:N ratio for protoplasm (1 14) When B_{12} administration was stopped balances gradually decreased to former levels. In animals on a constant intake containing 2.5 gm. N per day slight positive nitrogen balance occurred under the influence of B_{12} even though additional food was not available during this period. On a constant diet of oats and cabbage which furnished a low N intake, urinary excretion of nitrogen, urea, amino acids and phosphorus diminished during B_{12} administration. These results indicate that in the normal rabbit under these conditions increased utilization of nitrogen and phosphorus occurred."

Medical College of Virginia
Richmond, Va.

714 BÉNARD H., GAJDOS A., and GAJDOS-TÖRÖK, M.: *Continuous metabolism of haemoglobin in the red cells of peripheral blood*, Nature 167 989-990, June 16, 1951

Subcutaneous injection of a single dose of 15 mg. of folic acid or of 20 mcg of vitamin B_{12} induces in rabbits, in 6 to 24 hours, a notable erythrocytosis. But at the same time the hemoglobin value, instead of increasing, remains at its initial level, with a consequent marked lowering of the color index (0.80-0.93 to 0.53-0.75)

A concept of continuous metabolism of hemoglobin is outlined. The authors have observed the content of hemoglobin of the red cell to be variable during its life protoporphyrin derived from degradation of hemoglobin is not utilized for the resynthesis of hemoglobin instead new protoporphyrin has to be synthesized.

Paris, France

715 DE MELLO R. P *Effect of vitamin B_{12} folic acid and nicotinamide on urinary coproporphyrin excretion in photosensitized rabbits*, Proc. Soc. Exper Biol. & Med. 77 744-747 Aug. 1951.

The author has previously shown that the photodynamic effect of Rose Bengal and ultraviolet rays increases urinary coproporphyrin within 24 to 48 hours in rabbits. In the present paper the effect of vitamin B_{12}, folic acid, and nicotinic acid on this increase was studied. Folic acid (5, 15, or 45 mg.) and vitamin B_{12} (5, 10, 15 or 30 mcg.) were injected 10 minutes before the administration of Rose Bengal and nicotinamide was given in three doses totaling 200 to 300 mg. one dose was given before and two doses after administration of Rose Bengal. Folic acid in the lower doses partially and in the highest dose completely inhibited increase of urinary coproporphyrin after administration of Rose Bengal and exposure to ultraviolet rays. Vitamin B_{12} almost completely prevented the increase of urinary coproporphyrin. The combination of folic acid and B_{12} was no more effective than the substances individually Nicotinamide decreased the coproporphyrinuria caused by ultraviolet rays, but did not prevent it. Since these three substances are all intimately related to cellular metabolism, it seems highly likely that the increases of urinary coproporphyrin under the experimental conditions described are likewise so related, but whether to the metabolism of the bone marrow or to some other system of cells is not clear

University of Minnesota Hospital
Minneapolis, Minn.

716 KULWICH, R., STRUGLIA, L., and PEARSON P. B.: *The effect of coprophagy on the excretion of B vitamins by the rabbit*, J Nutrition 49 639-645 April 10, 1953.

Authors summary "Rabbits that are collared to prevent coprophagy or consumption of the soft feces excrete approximately 29% more feces than are recovered when coprophagy is not prevented. The difference is essentially accounted for by the soft feces excreted. The soft feces contain between three and 4 times more niacin and riboflavin per gram than do the hard feces. Pantothenic acid is about 6 times greater in the soft feces and vitamin B_{12} between two and three times the level

the hard feces. On the basis of the difference in the fecal excretion of the vitamins or their recovery in the feces of the rabbits while collared as compared with the amounts recovered while the animals were not collared, it can be calculated that coprophagy provides the rabbit with about 83% more niacin, 100% more riboflavin, 165% more pantothenic acid and 42% more vitamin B_{12} than would be available if the soft feces were not consumed.

"The amount of vitamin B_{12} recovered in the feces of rabbits fed a diet low in this vitamin was 221 fold greater than the intake. Values are given for the vitamin B_{12} content of the liver kidney and spleen of the rabbit."

U. S. Department of Agriculture
Washington, D. C.

717 NUTRITION REVIEWS: *The utilization of vitamin B_{12}*, Nutrition Reviews 10 219- July 1952 (abstr J Am. Dietet. A. 28 1078, Nov 1952)

"Data have seemed to indicate that vitamin B_{12} is apparently not absorbed in any large quantity in normal individuals. From recent evidence, however it appears that in dogs, absorption of the vitamin is substantially proved on the basis of blood levels. When orally administered to rats, the vitamin showed evidence of absorption and its level in the diet had a marked effect upon its quantity in the animals milk. In studying the influence of the diet as a whole upon the rat s absorption of vitamin B_{12}, it appeared that pork may contain a factor or factors that somehow interferes with normal utilization of the vitamin. Cobalt injections produced an expected increase in red blood cells but failed to influence the storage of vitamin B_{12} in the liver The polycythemia inducing action of cobalt cannot be explained on the basis of an increased formation of the vitamin in the rat. Present evidence indicates that absorption varies considerably and is affected by the general diet."

718. ROSENTHAL, H. L., and HAMPTON J K. *Absorption of vitamin B_{12} in dogs*, Federation Proc. 12 428, March 1953.

"A procedure to determine vitamin B_{12} activity in blood serum with *Lactobacillus leichmannii* (ROSENTHAL AND SARETT *J B C.* 199 433, 1952) was applied to the study of absorption of vitamin B_{12} from the intestinal tract of dogs. Crystalline vitamin B_{12} was placed in the ligated and unligated duodenum of anaesthetized dogs and samples of portal and peripheral blood were obtained over an eight hour period. In other dogs, vitamin B_{12} was placed in the stomach ligated at the pyloric sphincter Serum vitamin B_{12} activity of eleven fasted dogs was 0.25 ± 0.10 µg./ml. which is similar to that of normal human subjects, 0.20 ± 0.08 µg./ml. In two dogs with ligated duodenums given 50 µg. vitamin B_{12}/kg., serum activity increased from fasting values to maxima of 3.4 µg./ml. and 6.5 µg./ml. after 4 and 8 hours respectively With 10 µg./kg., serum activity increased slightly in one dog and rose to 0.95 µg./ml. in another In one dog with an unligated duodenum, maximum serum activity after 50 µg./kg. was 1.68 µg./ml. In two dogs with stomachs ligated, no rise in serum activity followed 50 µg./kg. In dogs subjected to operation but given no vitamin B_{12}, serum activity remained constant. Findings in portal blood followed the same pattern as in peripheral blood. These data show that vitamin B_{12} is absorbed primarily in the duodenum and not in the stomach."

Tulane University School of Medicine
New Orleans, La.

719 LANG, C. A., and CHOW B. F *Retention of vitamin B_{12} after administration to subjects of different ages*, Federation Proc. 11 88, March 1952.

"A small portion of the vitamin B_{12} administered parenterally to normal adult rats in dosages of 1 2 µg. appears in the urine, and the remainder is retained by certain tissues and concentrated in organs such as liver kidneys, and pancreas. Experiments were carried out to compare the urinary excretion and retention of this vitamin after injection into rats of different ages. For this purpose, 15 2-month-old and 16 14-month-old male rats raised on our stock diet fortified with 2% desiccated whole liver were placed in individual metabolism cages. They were divided into 4 sub-groups and given a single subcutaneous injection of crystalline vitamin B_{12} tagged with Co^{60} in dosages of 1-8 µg./rat. Urine specimens (0-24 and 24-28 hours after administration and in some instances pre-injection samples) were collected and assayed for vitamin B_{12} both by a microbiological method and by radioactivity measurement. Fourteen days after injection the animals were sacrificed, and the above-mentioned organs were removed for the determination of radioactivity after conversion into inorganic Co^{60} salts. Our results demonstrate 1) At a given dosage the excretion of vitamin B_{12} was essentially the same for both age groups if expressed as µg./animal but was significantly different if expressed as µg./100 gm. body weight. This is due to the fact that the average weight of the older animals (457 gm.) was approximately twice that of the young ones (248 gm.) 2) Retention was related both to dosage and to age. Similar excretion studies were made using human male subjects of 2 different age groups, namely 25-40 years of age and 65 or greater who were of essentially the same body weight. Our preliminary results again indicated that the young individuals excreted more."

Johns Hopkins University
Baltimore, Md

720. SIEBERT G., LANG K., and LANG H. *Investigations on the metabolism in cell nuclei. Vitamin B_{12} and cobalt in cell nuclei*, Biochem. Z. 321 543-548, May 1951.

The fact that the vitamin B_{12} and cobalt content of cell nuclei is very low and that following intravenous injection of Co^{60} the nuclei absorb only half as much cobalt as tissues, indicates that B_{12} and cobalt have no significant part in the metabolism of cell nuclei.

VITAMIN B_{12} CONTENT ACTIVITY

721 COUCH, J R., OLCESE, O., WITTEN, P W., and COLBY R. W: *Vitamin B_{12} content of blood from various species*, Am. J Physiol. 163 77-80, Oct. 1950.

The authors determined the vitamin B_{12} content of whole blood from a number of species as measured by Lactobacillus leichmannii 4749 Vitamin B_{12} activity for some of the species was determined also after autoclaving of the whole blood samples at pH 12. Values obtained are listed in the accompanying table

SOURCE OF BLOOD SAMPLES	B_{12} CONTENT (mcg./cc. blood) Before autoclaving	After autoclaving	B_{12} ACTIVITY (mcg./cc. blood) destroyed by autoclaving
human	0.8	—	—
dog	0.9	—	—
cotton rat	3.6	0.0	3.6
white rat	0.8	0.0	0.7
American albino mice	2.3	0.0	2.3
Swiss albino mice	1.2	0.0	1.2
calf (dry lot fed)	0.9	0.0	0.9
cow (pasture fed)	0.5	—	—
sheep	0.7	0.0	0.7
goat	0.7	0.0	0.7
pig	1.0	—	—
horse	2.1	—	—
rabbit	10.1	0.0	10.1
turtle	6.6	4.6	2.0
chick	5.6	4.8	1.0
turkey	5.3	3.8	1.5

It can be observed from the table that the vitamin B_{12} activity of blood in most of the species was destroyed by autoclaving at pH 12.0. In the turtle, chicken and turkey which had a vitamin B_{12} activity appreciably higher than that of the other species tested except for the rabbit, only a portion of the B_{12} activity was destroyed. This is believed to be due to the fact that these animals have nucleated erythrocytes: therefore a part of the L. leichmannii activity is due to thymidine. The exceptionally high B_{12} content of rabbit blood is believed attributable to the habit of nocturnal coprophagy

Texas Agricultural Experiment Station
College Station, Texas

722. PFANDER, W H., DIETRICH, L. S., MONSON W J., HARPER, A. E., and ELVEHJEM, C. A. *Citrovorum factor vitamin B_{12} and folic acid activity of whole blood of several species* Proc. Soc. Exper Biol. & Med. 79 219-221, Feb. 1952.

The citrovorum factor (LCF) vitamin B_{12}, and folic acid (FCA) content of whole blood of several species (calf, chicken, sheep, ox, and rat in various stages of B_{12} depletion) was studied. Little or no free LCF active material was demonstrated, but demonstrable amounts of free Streptococcus faecalis activity were found. Release of LCF and FCA during autolysis was dependent on the pH of the reaction mixture optimum values were obtained at pH 4.5 or 6.5 to 7.0, depending on the species studied. LCF was low in hyperthyroid vitamin B_{12}-deficient rats but levels similar to those in normal rats after fasting were found in similar rats given 3 mcg. of vitamin B_{12} a day; FCA activity was not appreciably changed by administration of vitamin B_{12} but the B_{12} level was significantly increased.

University of Wisconsin
Madison, Wis.

723 DOCTOR, V M., and COUCH, J R. *Bioautographic analysis of blood for growth factors active for L. leichmannii 4797* Proc. Soc. Exper Biol. & Med. 81 222 224, Oct. 1952.

Aqueous extracts of autolyzed blood samples from various species were chromatographed on paper strips and analyzed by bioautographic procedures.

Chicks

724 COUCH, J R., OLCESE, O., and GERMAN H. L. *Vitamin B_{12} content of chick tissues as influenced by diet*, Federation Proc. 9 355, March 1950.

725 COUCH, J R., and OLCESE, O *The vitamin B_{12} content of chick tissues as influenced by the diet*, J Nutrition 42 337-346, 1950.

726. DARBY H. H. *Metabolism of cobalt 56 in the hen's ovary* Federation Proc. 9 356, March 1950.

"With the publication of the fact that cobalt is incorporated in vitamin B_{12} and that B_{12} is found in the droppings of chickens, it became of interest to study the fate of cobalt fed to hens. It had already been shown that increased growth could be obtained by injecting small amounts of B_{12} into developing eggs. This would lead one to believe that there was a shortage of B_{12} in the average hen's diet. Cobalt 56 was fed in pellet form to 2 hens and the eggs collected for some 3 weeks thereafter The eggs were tested as a whole first for radioactivity and then they were separated into shell, albumen, and yolk. The amount of cobalt in each fraction was ascertained. The shell and the albumen followed a typical excretory pattern. There was a rise of radioactive cobalt in the shell and the albumen on the 4th-5th day followed by a fall to almost zero by the 10th day The rise of radioactive cobalt in the yolk, however was somewhat slower and although there was a high level for some 10 days, no fall of cobalt was found similar to that in the albumen and the shell. It leads one to believe that the yolk of the egg follows a different pattern of metabolism than does that of the shell and the albumen. From these experiments it seems that cobalt once laid down in the yolk is retained in these formative eggs."

Carnegie Institution of Washington
Washington, D. C.

727 JACKSON, J T., MACHLIN L. J., BRANDENBURGER, E. A., KELLOGG, W L., and DENTON C. A.: *Retention of Co 60 labeled vitamin B_{12} in chickens*, Proc. Soc. Exper Biol. & Med. 83 221 222, June 1953.

Authors summary: "The results indicate that over 30% of the injected Co 60 activity is retained for as long as 12 weeks in chicks hatched from eggs injected with the

the hard feces. On the basis of the difference in the fecal excretion of the vitamins or their recovery in the feces of the rabbits while collared as compared with the amounts recovered while the animals were not collared, it can be calculated that coprophagy provides the rabbit with about 83% more niacin, 100% more riboflavin, 165% more pantothenic acid and 42% more vitamin B_{12} than would be available if the soft feces were not consumed.

"The amount of vitamin B_{12} recovered in the feces of rabbits fed a diet low in this vitamin was 221 fold greater than the intake. Values are given for the vitamin B_{12} content of the liver kidney and spleen of the rabbit."

U. S. Department of Agriculture
Washington, D. C.

717 NUTRITION REVIEWS: *The utilization of vitamin B_{12}*, Nutrition Reviews 10 219- July 1952 (abstr J Am. Dietet. A. 28 1078, Nov 1952)

"Data have seemed to indicate that vitamin B_{12} is apparently not absorbed in any large quantity in normal individuals. From recent evidence, however it appears that in dogs, absorption of the vitamin is substantially proved on the basis of blood levels. When orally administered to rats, the vitamin showed evidence of absorption and its level in the diet had a marked effect upon its quantity in the animals milk. In studying the influence of the diet as a whole upon the rat s absorption of vitamin B_{12}, it appeared that pork may contain a factor or factors that somehow interferes with normal utilization of the vitamin. Cobalt injections produced an expected increase in red blood cells but failed to influence the storage of vitamin B_{12} in the liver The polycythemia-inducing action of cobalt cannot be explained on the basis of an increased formation of the vitamin in the rat. Present evidence indicates that absorption varies considerably and is affected by the general diet."

718. ROSENTHAL, H. L., and HAMPTON J K.: *Absorption of vitamin B_{12} in dogs* Federation Proc. 12 428, March 1953

"A procedure to determine vitamin B_{12} activity in blood serum with *Lactobacillus leichmannii* (ROSENTHAL AND SARETT *J B. C.* 199: 433, 1952) was applied to the study of absorption of vitamin B_{12} from the intestinal tract of dogs. Crystalline vitamin B_{12} was placed in the ligated and unligated duodenum of anaesthetized dogs and samples of portal and peripheral blood were obtained over an eight hour period. In other dogs, vitamin B_{12} was placed in the stomach ligated at the pyloric sphincter Serum vitamin B_{12} activity of eleven fasted dogs was 0.25 ± 0.10 µg./ml. which is similar to that of normal human subjects, 0.20 ± 0.08 µg./ml. In two dogs with ligated duodenums given 50 µg. vitamin B_{12}/kg., serum activity increased from fasting values to maxima of 8.4 µg./ml. and 6.5 µg./ml. after 4 and 8 hours respectively With 10 µg./kg., serum activity increased slightly in one dog and rose to 0.95 µg./ml. in another In one dog with an unligated duodenum, maximum serum activi 50 µg./kg. was 1.68 µg./ml. In two dogs with s ligated, no rise in serum activity followed 50 µg. dogs subjected to operation but given no vitam serum activity remained constant. Findings in blood followed the same pattern as in periphera These data show that vitamin B_{12} is absorbed pr in the duodenum and not in the stomach."

Tulane University School of Medicine
New Orleans, La.

719 LANG, C. A., and CHOW B. F *Retention mun B_{12} after administration to subjects of d ages*, Federation Proc. 11 88, March 1952

"A small portion of the vitamin B_{12} admi parenterally to normal adult rats in dosages of appears in the urine, and the remainder is retai certain tissues and concentrated in organs such a kidneys, and pancreas. Experiments were carried compare the urinary excretion and retention of th min after injection into rats of different ages. F purpose, 15 2-month-old and 16 14-month-old m raised on our stock diet fortified with 2% des whole liver were placed in individual metabolism They were divided into 4 sub-groups and given a subcutaneous injection of crystalline vitamin B_{12} with Co^{60} in dosages of 1-8 µg./rat. Urine sp (0-24 and 24-28 hours after administration and i instances pre-injection samples) were collected a sayed for vitamin B_{12} both by a microbiological and by radioactivity measurement. Fourteen day injection the animals were sacrificed, and the abo tioned organs were removed for the determinat radioactivity after conversion into inorganic Co Our results demonstrate 1) At a given dosage the tion of vitamin B_{12} was essentially the same for b groups if expressed as µg./animal but was signi different if expressed as µg./100 gm. body weigh is due to the fact that the average weight of the animals (457 gm.) was approximately twice that young ones (248 gm.) 2) Retention was related dosage and to age. Similar excretion studies wer using human male subjects of 2 different age namely 25-40 years of age and 65 or greater wh of essentially the same body weight. Our preli results again indicated that the young individuals e more."

Johns Hopkins University
Baltimore, Md.

720. SIEBERT G., LANG K., and LANG H. *I gations on the metabolism in cell nuclei. V B_{12} and cobalt in cell nuclei*, Biochem. Z. 321 548, May 1951.

The fact that the vitamin B_{12} and cobalt con cell nuclei is very low and that following intra injection of Co^{60} the nuclei absorb only half as cobalt as tissues, indicates that B_{12} and cobalt h significant part in the metabolism of cell nuclei.

VITAMIN B_{12} CONTENT ACTIVITY

721 COUCH, J R., OLCESE, O., WITTEN P W., and COLBY R. W *Vitamin B_{12} content of blood from various species*, Am. J Physiol. 163 77-80 Oct. 1950.

The authors determined the vitamin B_{12} content of whole blood from a number of species as measured by Lactobacillus leichmannii 4749 Vitamin B_{12} activity for some of the species was determined also after autoclaving of the whole blood samples at pH 12. Values obtained are listed in the accompanying table:

SOURCE OF BLOOD SAMPLES	B_{12} CONTENT (mcg./cc. blood)		B_{12} ACTIVITY (mcg./cc. blood) destroyed by autoclaving
	Before autoclaving	After autoclaving	
human	0.8	—	—
dog	0.9	—	—
cotton rat	3.6	0.0	3.6
white rat	0.8	0.0	0.7
American albino mice	2.3	0.0	2.3
Swiss albino mice	1.2	0.0	1.2
calf (dry lot fed)	0.9	0.0	0.9
cow (pasture fed)	0.5	—	—
sheep	0.7	0.0	0.7
goat	0.7	0.0	0.7
pig	1.0	—	—
horse	2.1	—	—
rabbit	10.1	0.0	10.1
turtle	6.6	4.6	2.0
chick	5.6	4.8	1.0
turkey	5.3	3.8	1.5

It can be observed from the table that the vitamin B_{12} activity of blood in most of the species was destroyed by autoclaving at pH 12.0 In the turtle, chicken and turkey which had a vitamin B_{12} activity appreciably higher than that of the other species tested except for the rabbit, only a portion of the B_{12} activity was destroyed. This is believed to be due to the fact that these animals have nucleated erythrocytes: therefore a part of the L. leichmannii activity is due to thymidine. The exceptionally high B_{12} content of rabbit blood is believed attributable to the habit of nocturnal coprophagy

Texas Agricultural Experiment Station
College Station, Texas

722. PFANDER, W H., DIETRICH, L. S., MONSON W J., HARPER, A. E., and ELVEHJEM, C. A. *Citrovorum factor vitamin B , and folic acid activity of whole blood of several species*, Proc. Soc. Exper Biol. & Med. 79 219-221 Feb. 1952.

The citrovorum factor (LCF) vitamin B_{12}, and folic acid (PGA) content of whole blood of several species (calf, chicken, sheep, ox, and rat in various stages of B_{12} depletion) was studied. Little or no free LCF active material was demonstrated, but demonstrable amounts of free Streptococcus faecalis activity were found. Release of LCF and PGA during autolysis was dependent on the pH of the reaction mixture optimum values were obtained at pH 4.5 or 6.5 to 7.0, depending on the species studied. LCF was low in hyperthyroid vitamin B_{12}-deficient rats but levels similar to those in normal rats after fasting were found in similar rats given 3 mcg. of vitamin B_{12} a day PGA activity was not appreciably changed by administration of vitamin B_{12} but the B_{12} level was significantly increased.

University of Wisconsin
Madison, Wis.

723 DOCTOR, V M., and COUCH, J R. *Bioautographic analysis of blood for growth factors active for L. leichmannii 4797* Proc. Soc. Exper Biol. & Med. 81 222-224 Oct. 1952.

Aqueous extracts of autolyzed blood samples from various species were chromatographed on paper strips and analyzed by bioautographic procedures.

Chicks

724 COUCH, J R., OLCESE, O., and GERMAN H. L. *Vitamin B_{12} content of chick tissues as influenced by diet*, Federation Proc. 9 355, March 1950.

725. COUCH, J R., and OLCESE, O : *The vitamin B_{12} content of chick tissues as influenced by the diet*, J Nutrition 42 337-346, 1950.

726. DARBY H. H. *Metabolism of cobalt 56 in the hen's ovary* Federation Proc. 9 356, March 1950.

"With the publication of the fact that cobalt is incorporated in vitamin B_{12} and that B_{12} is found in the droppings of chickens, it became of interest to study the fate of cobalt fed to hens. It had already been shown that increased growth could be obtained by injecting small amounts of B_{12} into developing eggs. This would lead one to believe that there was a shortage of B_{12} in the average hen's diet. Cobalt 56 was fed in pellet form to 2 hens and the eggs collected for some 3 weeks thereafter The eggs were tested as a whole first for radioactivity and then they were separated into shell, albumen, and yolk. The amount of cobalt in each fraction was ascertained. The shell and the albumen followed a typical excretory pattern. There was a rise of radioactive cobalt in the shell and the albumen on the 4th-5th day followed by a fall to almost zero by the 10th day The rise of radioactive cobalt in the yolk, however was somewhat slower and although there was a high level for some 10 days, no fall of cobalt was found similar to that in the albumen and the shell. It leads one to believe that the yolk of the egg follows a different pattern of metabolism than does that of the shell and the albumen. From these experiments it seems that cobalt once laid down in the yolk is retained in these formative eggs."

Carnegie Institution of Washington
Washington, D. C.

727 JACKSON, J T., MACHLIN L. J., BRANDENBURGER, E. A., KELLOGG W L., and DENTON C. A. *Retention of Co 60 labeled vitamin B_{12} in chickens*, Proc. Soc. Exper Biol. & Med. 83 221-222, June 1953.

Authors summary "The results indicate 30% of the injected Co 60 activity is retained as 12 weeks in chicks hatched from eggs inj

radiovitamin. The total B_{12} content of the chick as measured microbiologically roughly parallels Co 60 activity until 6 weeks of age, after which there is a decided increase in the total B_{12} content of the chicks."

U. S. Department of Agriculture
Beltsville, Md.

Rats, Mice

728. LEWIS, U J., REGISTER, U. D., and ELVEHJEM, C. A.: *Vitamin B_{12} content of various organs and tissues of the rat*, Proc. Soc. Exper Biol. & Med. 71 509-511, July 1949

Assays are reported showing no appreciable amount of vitamin B_{12} in the liver heart, small intestine, or muscle of rats kept on a basal corn-soybean diet for six weeks the kidney contained 0.04 microgram of vitamin B_{12} per gram. Addition of vitamin B_{12} to the diet increased the amount of B_{12} in all the organs and tissues tested. The amounts, in micrograms per gram, were as follows kidney 0.17 liver 0.08; heart, 0.07; intestine, 0.04 spleen, no measurable amount muscle, trace.

729 CUTHBERTSON W F J., FREE, A. A., and THORNTON D M.: *Distribution of radioactive cobalt in the rat*, Brit. J Nutrition 4 42-48, 1950.

Cobalt is preferentially concentrated in the liver kidney spleen (and pancreas)

730. RICHARDSON L. R., WITTEN P W., and COUCH, J R.: *Diet of mother and vitamin B_{12} content of tissues of infant rats*, Proc. Soc. Exper Biol. & Med. 76 265-267 Feb. 1951.

Experiments were conducted to determine the effect of various vitamin B_{12} supplements added to the mothers diet on the B_{12} content of the liver spleen, heart, and kidney of 28 day old rats. The mothers were fed the B_{12} products from 28 days of age until at least four litters were produced.

Group	Vitamin B_{12} (mcg./Kg. feed)	Average mμg. B_{12}/100 Gm. of Tissues of Young			
		Liver	Spleen	Heart	Kidney
1	None	4.1	7.7	1.7	11.3
2	36 (APF No. 3)	6.2	11.4	4.2	20.6
3	6 (APF concentrate)	6.4	11.7	2.9	11.5
4	27 (Liver "L")	12.9	12.8	3.4	20.6

These results show that when Liver "L" was added to the basal ration the amount of vitamin B_{12} in the liver spleen and heart approximately doubled. The diet of the mother had no significant effect on the B_{12} content of the kidney The kidney and spleen contained slightly more vitamin B_{12} than the liver In comparison to other tissues, the heart contained a relatively small amount of B_{12}.

Agricultural and Mechanical College of Texas
College Station, Texas

731. CHOW B. F., ROSENBLUM, C., SILBER, R. H., WOODBURY D T., YAMAMOTO R., and LANG C. *Oral administration of vitamin B_{12} containing cobalt[60] to rats*, Proc. Soc. Exper Biol. & Med. 76 393-395, Feb. 1951.

The fate of orally administered vitamin B_{12} in the body was studied by means of a vitamin B_{12} preparation made radioactive by cobalt[60] Daily collections of urine and feces were made for two days before and three or four days after the administration to rats of a single oral dose of 0.89 mg. of radioactive B_{12}. The animals were sacrificed on the fifth day and the kidneys, liver spleen, brain, testes, and hearts removed. It was found that the amount of vitamin B_{12} excreted in the urine was very low compared to the large doses administered—never more than 5% The greatest elimination occurred within the first 24 hours. The vitamin B_{12} content of the feces was high; in one instance, equivalent to 65% of the amount added to the diet. In the kidneys and liver radioactivity was equivalent to 0.5% of administered B_{12}. Radioactivity of the blood, though small, indicated the passage of B_{12} through the blood stream. The quantities listed for the remaining organs were doubtful. The dose of 0.89 mg. of vitamin B_{12} is far in excess of physiologic requirements, and, therefore, its presence in the urine, even in low values, proves that intestinal absorption takes place upon oral administration.

Johns Hopkins University
Baltimore, Md.
Merck Research Laboratories, and
Merck Institute for Therapeutic Research
Rahway N J

732. CHOW B. F., BARROWS, L., and LING, C. T.: *The distribution of radioactivity in the organs of the fetus or of young rats borne by mothers injected with vitamin B_{12} containing Co^{60}* Arch. Biochem. & Biophysics 34 151 157 Nov 1951.

Vitamin B_{12} is apparently transferred placentally After administration of vitamin B_{12} containing Co^{60} to pregnant rats, the vitamin was found in small amounts in liver and kidneys, in even lesser amounts in intestines and in insignificant amounts in spleen and heart of mother rats. The carcasses of the newborn contained large amounts.

733 ELLINGSON R. C., MUELLER, A. J., COX, W M., JR., and CHOW B. F: *Appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B_{12} containing cobalt[60]* Federation Proc. 11 441, March 1952.

"With the availability of vitamin B_{12} containing cobalt[60] and the development of a satisfactory method of milking small animals it became possible to determine the appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B_{12} Two groups of lactating rats were given 300 or 500 mμ. of vitamin B_{12} orally and a 3rd group 300 mμ. subcutaneously The rats were milked at various intervals (2 16 hours) thereafter and the radioactivity of the milk measured. Each of the 54 rats was milked only once; the volume was 0.5-4.0 cc./rat. No radioactivity was found in milk of 28 of the animals receiving vitamin B_{12} orally and only traces in milk of the other 10. Large amounts of radioactivity were found in milk of 15 rats receiving the vitamin subcutaneously a trace was found in the milk of one. There was no correlation between the quantity of radioactivity in the milk and the interval between administration of the vitamin and milking of the rats. The mothers were returned to their young and subse-

quently 1 rat from each litter was killed. The radioactivity of the kidneys, liver and carcass was determined. None could be detected in these tissues. Assuming radioactivity of the milk to be a measure of vitamin B_{12} at least 3.5 times as much B_{12} is secreted/cc. of milk by rats receiving the vitamin subcutaneously than by those receiving it orally"

Mead Johnson Research Laboratories
Evansville, Ind.
Johns Hopkins University
Baltimore, Md.

734 HARTE, R. A., CHOW B. F., and BARROWS, L.: *Disappearance of radioactivity from various organs of normal adult rats following subcutaneous injection of tagged vitamin B_{12}*, Federation Proc. 11 446, March 1952.

"Following the earlier demonstration that subcutaneously injected radioactive (Co^{60}) vitamin B_{12} led to retention of radioactivity in the tissues of various organs (kidney liver muscle, etc.) even after 2 weeks, estimation of the rate of disappearance of such concentrations of radioactivity becomes of interest. Thirty healthy adult rats, both males and females, from the stock colony were injected, subcutaneously with a single dose of 2 µg. of tagged vitamin B_{12} (specific activity 60 µc./mg.) The animals were kept in individual metabolism cages. Urine specimens were collected after 1 7 and 14 days and assayed for vitamin B_{12} by the microorganism adsorption technic of Davis and Chow Groups of 5 animals (2 males and 3 females) were sacrificed at intervals and various organs and tissues carefully dissected out. Following acid digestion, radioactivity of the cobalt salts was determined. The findings indicate that after the first day more radioactivity is localized in the kidneys than elsewhere, but this amount tended to decrease sharply with time. Lesser absolute amounts but much higher concentrations of activity were found in the pancreas. The amounts of radioactivity in liver pancreas, muscle and spleen remained essentially constant or even increased with time. Vitamin B_{12} in liver is apparently associated with the protein moiety since the total amount of radioactivity remains constant but the concentration rises when the liver glycogen is mobilized as a result of caloric deprivation."

Sharp and Dohme Inc.
West Point, Pa.
Johns Hopkins University
Baltimore, Md.

735 HARTE, R. A., CHOW B. F., and BARROWS, L. *Storage and elimination of vitamin B_{12} in the rat*, J Nutrition 49 669-678, April 10, 1953

Authors summary: "Data are presented on the storage of radioactivity in selected target organs following administration to rats of Co^{60} containing vitamin B_{12}. Noteworthy is the persistence, up to three months, of significant storage in the pancreas.

"The effects on storage in selected organs of flooding of the host with the vitamin have been studied. In this instance some evidence suggestive of flushing out in liver and pancreas has been found, but the storage of radioactivity in the kidney appears to be unaffected.

"The state of saturation of the host has been shown to play a role in the extent of storage of a tagged dose in the liver pancreas and kidneys. In the first two organs, storage is less in the saturated animal m the kidneys, greater storage is seen."

Sharp and Dohme, Inc.
West Point, Pa.
Johns Hopkins University
Baltimore, Md.

736. SWENDSEID M. E., BETHELL, F H., and ACKERMANN W W *The intracellular distribution of vitamin B_{12} and folinic acid in mouse liver* J Biol. Chem. 190 791 798, June 1951.

The folic acid activity of mouse liver is due to its folinic acid content. Under standard dietary conditions, folic acid is absent from the liver or present in very small amounts. The intracellular distribution patterns of vitamin B_{12} and folinic acid in liver have been determined. Vitamin B_{12} is concentrated in the mitochondria, whereas folinic acid appears to be equally distributed in the particulate and supernatant fractions.

University of Michigan
Ann Arbor Mich.

737 MOVITT E. R. *Megaloblastic erythropoiesis in patients with cirrhosis of the liver* Blood 5 468-477, May 1950.

Case reports are given of 3 patients with cirrhosis of the liver macrocytic anemia, and megaloblastic proliferation in the bone marrow These patients gave histories indicating grossly deficient diets for many years. In each case free hydrochloric acid was present in the gastric juice, which was thought to exclude the possibility of coexisting addisonian pernicious anemia. Vitamin B_{12} was used in the treatment of one patient remission followed a single dose of 150 mcg.

Veterans Administration Hospital
Oakland, Calif.

738. STEIGMANN F *Advances in the management of jaundice experiences with 500 jaundiced patients* J.A.M.A. 144 1076-1081 Nov 25, 1950.

In the discussion of dietary treatment it is stated that patients with jaundice should receive supplementary vitamins in addition to a diet high in calories and vitamins. The plasma of jaundiced patients is low in vitamin A. Doses of 50,000 to 100 000 units of vitamin A in oil are most likely to cause a rise of the plasma vitamin A level. The recent preparation of vitamin A in aqueous solution is better absorbed and more economical because a dose with about one-half the unit value of the oily solution will similarly raise the vitamin A level of the plasma. Patients who cannot take anything by mouth should be given the vitamin A parenterally preferably in the form of vitamin A palmitate in aqueous dispersion. Jaundiced patients should receive vitamin K when their prothrombin levels are low and they also require large doses of vitamin C (500 to 1,000 mg. daily) because it has a favorable effect on carbohydrate and protein metabolism and prevents fat deposition. The B complex vitamins are very important to jaundiced patients. Natural B complex is preferable to individual synthetic B complex factors because the former contains unknown factors which may be required. Vitamin B_{12} should be given in large doses (30 mcg. daily or more) to patients with liver disease.

University of Illinois College of Medicine
Chicago, Ill.

739 CAMPBELL, R. E., and PRUITT F W *Vitamin B_{12} in the treatment of viral hepatitis a preliminary report,* Am. J M. Sc. 224 252-262, Sept. 1952.

Authors summary "Three groups of 100 patients each, who had acute viral hepatitis, have been compared with respect to clinical course, duration of illness and incidence of relapse. The groups were comparable as regards age, race, duration of illness and degree of icterus. Group I received the conventional treatment consisting of a high-protein, high-carbohydrate, moderate-fat diet, bed rest, multivitamins and brewer s yeast. Group II received the same diet and bed rest without added vitamins or brewer s yeast. Group III received the same treatment as Group II but with the addition of 30 mcg. of vitamin B_{12} by mouth daily for the first 5 days of hospitalization. The following results were observed:

"1 The patients who received vitamin B_{12} had a more rapid return to normal appetite and liver size than did the patients in the other two groups.

"2. The total serum bilirubin values of al[...] tients in the vitamin B_{12} group were norma[...] 10th week of illness, whereas 18 weeks were r[...] Group II, and 24 weeks by Group I.

"3. The mean duration of illness, calculat[...] date of the onset of the first symptom to the da[...] function tests became normal and remained sc[...] days for the patients who received vitamin [...] days for those who received the prescribed di[...] rest only and 56.7 days for those who receive[...] ventional regimen for hepatitis.

"4. There were 5 instances of relapse in [...] group, 4 such instances in the group receivin[...] bed rest and only 2 among the patients given vi[...]

"5. Experimental work links vitamin B_{12} [...] protein metabolism and, hence, liver repair [...] a lipotropic agent is further evidence of its i[...] in liver diseases. The relationship of vitamin I[...] acid in this process is considered.

"6. Possible objections to the validity of [...] obtained herein are raised, but it is conclude[...] results are sufficiently suggestive as to warra[...] investigation."

Sixth Division Hospital (Hepatitis Center) and
Office of Medical Consultants, CPO, FEC
San Francisco, Calif.

740. DROUET P L., WOLFF KARLIN and [...] G *Vitamin B_{12},* J.A.M.A. 146 493, Ju[...] (in Foreign Letters, Paris)

Studies were made of vitamin B_{12} levels [...] and animal liver in vivo. In the ox the levels ra[...] 0.5 to 1.5 mcg. per Gm. of liver parenchyma. [...] taken from human corpses revealed a B_{12} cont[...] to 0 7 mcg. per Gm. of body tissue. In 6 pati[...] various diseases, liver biopsies were done an[...] content of the hepatic tissue varied from 0.47 [...] mcg Vitamin B_{12} hepatic levels were studied i[...] with pernicious anemia before and after treatm[...] patients, before treatment, a considerable fa[...] hepatic B_{12} levels (0.023, 0.037 0.041 0.071 [...] mcg.) was noted after treatment with a liver [...] with vitamin B_{12} in a dosage of 30 to 50 mcg. [...] considerable rise in the hepatic vitamin B_{12} [...] curred, the increased levels being two to five tim[...] than the pretreatment levels.

741 GIRDWOOD R. H. *Megaloblastic* [...] Lancet 2 1068, Dec. 8, 1951 (in Soc. P[...]

It was reported that by bacteriological m[...] had been found that normal liver tissue (obtai[...] mortem) contained much folinic acid, but no f[...] in a case of pernicious anemia where the pat[...] died from another cause after receiving only a s[...] of vitamin B_{12}, folic and folinic acid were p[...] equal proportions. No vitamin B_{12} had been [...] the tissues of an untreated pernicious anemia pat[...] had died there was no evidence of an inhibitor [...] tissues since added vitamin B_{12} could be recov[...] tact.

ANIMAL STUDIES

REGENERATION STORAGE, NECROSIS

742. DENTON R. W., and IVY A. C. *Effect of feeding liver on the rate of regeneration of the liver in partially hepatectomized rats* Am. J Physiol. 152 460-464, Feb. 1948.

"Liver tissue, either because it contains a high quality protein or some as yet undetermined growth-promoting factor other than thiamine or the anti-pernicious anemia factor facilitates the rate of regeneration of the liver in the partially hepatectomized rat."

743. STERN J R., TAYLOR, M. W., and RUSSELL, W C. *Relation of vitamin B_{12} to liver basophilia*, Proc. Soc. Exper Biol. & Med. 70 551-552, March 1949

Growth of weanling white rats was stimulated by inclusion of vitamin B_{12} in the diet at a level of 440 mcg./Kg. Whole dried liver also stimulated growth. Rats that received neither supplement grew poorly and showed little or no liver basophilia, whereas those receiving B_{12} or liver grew well and showed considerable cytoplasmic basophilia in their liver cells.

Rutgers University
New Brunswick, N J

744 VARS, H. M., KARN G M., and FERGUSON C. C.: *Vitamin B_{12} and liver protein regeneration*, Federation Proc. 9 373-374 March 1950.

"Adult male rats reared on a Purina diet were fed a nonprotein diet for 2 weeks and subjected to a 70% partial hepatectomy During a 2 week postoperative period suitable groups were fed diets containing no protein, 10 or 18% casein and 10% wheat gluten, with and without the subcutaneous or intraperitoneal injection of a total of 4-10γ of Cobione. There were no essential differences between treated and untreated groups in the N-balance, food intake, weight restoration and liver protein regeneration. A similar experiment was conducted with rats that had been fed a soya protein diet, free of animal protein factor for a period of 8-13 months. After 2 weeks on a non-protein diet and partial hepatectomy they were fed the same soya diet for 14 days, with and without injection of Cobione. The ad libitum-fed B_{12} treated rats regained more in body weight and regenerated as much liver protein (slightly less positive N-balance) than the untreated controls. The B_{12} treated controls, pair fed to the untreated controls regained the same amount of body weight but regenerated less liver protein and had a smaller positive N-balance. Under experimental conditions employed vitamin B_{12} has evidenced little or no stimulatory effect in facilitating liver protein regeneration."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

745 GYÖRGY P., and ROSE, C. S. *Effect of vitamin B_{12} on experimental hepatic injury* Proc. Soc. Exper Biol. & Med. 73 372-375, March 1950.

Authors summary "Vit. B_{12} (1γ daily by mouth) had no effect on the development of massive hepatic necrosis in rats fed experimental rations with yeast as sole source of protein.

"Vit. B_{12} (0.5γ daily by mouth) has shown significant lipotropic activity in rats fed a low protein–low fat ration. Under the experimental conditions chosen methionine was more effective than vitamin B_{12} in preventing fat deposition in the liver A low protein–high fat diet seemed to interfere with the lipotropic effect of B_{12}."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

746. DIETRICH, L. S., MONSON W J., and ELVEHJEM, C. A. *Observations on a relationship between vitamin B_{12}, folic acid and the citrovorum factor* Proc. Soc. Exper Biol. & Med. 77 93-96, May 1951

Studies of the relationship between vitamin B_{12}, folic acid (PGA) and the Leuconostoc citrovorum factor (LCF) are presented. In chicks deficient in PGA and vitamin B_{12}, the intramuscular injection of low levels of B_{12} (0.1 or 0.3 mcg. per day) increased the PGA stored in the liver but higher levels (0.5 or 1.0 mcg.) suppressed liver storage of PGA. Oral administration of 0.3 mcg. of vitamin B_{12} gave results similar to those obtained by intramuscular injection of the same amount.

When the vitamin B_{12}-deficient chicks received ample PGA, the oral administration of 0.5 mcg. of vitamin B_{12} per day produced a marked increase of both LCF and PGA levels in the liver while parenteral administration depressed the storage of PGA without changing that of LCF Oral administration produced slightly lower levels of liver storage of vitamin B_{12} than did parenteral administration of the vitamin.

Further experiments in chicks deficient in both PGA and vitamin B_{12} showed similar levels of this vitamin in the liver regardless of the route of administration. The liver level of PGA was lowered while that of LCF remained unchanged. Studies of liver slices from chicks given B_{12} by injection showed decrease in the amount of PGA converted to LCF this inhibition was overcome by the addition of ascorbic acid to the flask in which the determination was made. The amount of PGA converted to LCF was shown, with or without the in vitro use of ascorbic acid, to be significantly increased in chicks given vitamin B_{12} orally

These data demonstrate that vitamin B_{12} plays some role in the conversion of PGA to LCF but whether its action is a specific vitamin action or an indirect action is unknown. Various theories are briefly discussed.

University of Wisconsin
Madison, Wis.

747 GYÖRGY P., STOKES J., JR., GOLDBLATT H., and POPPER, H. *Antimicrobial agents in the prevention of dietary hepatic injury (necrosis, cirrhosis) in rats*, J Exper Med. 93 513-522, June 1951

Authors summary: "The effect of various antimicrobial agents, such as aureomycin, terramycin, streptomycin, chloromycetin, penicillin, polymyxin, and sulfaguandine on the development of massive dietary necrosis of the liver in rats has been studied. Delay in the production of hepatic necrosis was obtained from aureomycin and, to a lesser extent, from terramycin and

Indication of temporary protection was shown by sulfaguanidine, whereas chloromycetin, polymyxin, and penicillin were not protective.

"B_{12}, added alone, or in combination with aureomycin, to the basal experimental diet had no influence on the development of hepatic necrosis. A combination of pectin with streptomycin enhanced the protective effect of the antibiotic.

"All the antimicrobial agents tested, without relation to their effect on hepatic necrosis, produced temporary stimulation of growth in the experimental animals.

"The beneficial effect of aureomycin was not limited to the delay of hepatic necrosis but manifested itself also in the prevention of hepatic cirrhosis in rats fed a low protein (casein)–high fat diet. In contrast to control animals showing the usual combination of cirrhosis and renal changes, the rats receiving supplements of aureomycin were free of both cirrhosis and renal changes. The rats receiving aureomycin took more food in and gained weight.

"No microscopic alterations were seen in the pancreas of the control rats with cirrhosis.

"In both groups of experiments (necrosis and cirrhosis) the antimicrobial agents, with the exception of penicillin, were given mixed with the food. Their possible effect on the intestinal flora is discussed."

748. ABELL, M. R., and BEVERIDGE, J. M. R. *Studies on hepatic necrosis induced by dietary means IV Conditions affecting the production and prevention of massive liver necrosis*, A.M.A. Arch. Path. 52 428-440, Nov 1951.

Authors summary in part "In an attempt to elucidate further the factors involved in the production of massive liver necrosis in rats fed diets low in methionine, cystine and alpha-tocopherol, experimental conditions have been varied with regard to initial weight and sex of the test animal and with regard to various dietary supplements.

"The inclusion of cod liver oil greatly increased the necrogenic quality of the basal diet.

"Of the lipotropic and antilipotropic substances tested, choline, inositol, cholesterol, and cystine, only the last had any effect on the development of liver necrosis. This substance displayed its well-known prophylactic action.

"In confirmation of previous reports, alpha-tocopherol, like cystine, prevented the development of hepatic damage. A similar but definite effect was noted for synthetic vitamin K (menadione)

"Sulfaguanidine and aureomycin both caused definite amelioration of the necrogenic property of the basal diet.

"With the exception of vitamin B_{12} alterations in the vitamin supplement had no effect. In the case of B_{12} there appeared to be a slight increase in the time required for death due to liver necrosis, but this difference could not be shown to be of statistical significance. "

University of Western Ontario
London, Ontario, Canada

749 SCHEID H. E., ANDREWS M. M., and SCHWEIGERT B. S. *Liver storage of vitamin B_{12} by the rat*, Proc. Soc. Exper Biol. & Med. 78 558-560, Nov 1951

Storage of vitamin B_{12} in the liver of the rat was studied by determining the vitamin B_{12} potency for the growth of Lactobacillus leichmannii and Euglena gracilis of livers from rats fed different levels of vitamin B_{12}. The vitamin B_{12} potency was shown to increase as the level in the diet of the rats was increased. The values were only 1/10 to 1/20 those in beef liver however Unlike that of beef liver the vitamin B_{12} potency of rat liver was not reduced appreciably by alkali treatment. The vitamin B_{12} potency remaining in rat livers after alkali treatment may be due to the presence of alkali stable forms of vitamin B_{12} as well as to desoxyribosides. The weight of the liver in proportion to body weight was greater in rats on a vitamin B_{12}-deficient diet than in those on the optimal diet.

American Meat Institute Foundation, and
The University of Chicago
Chicago, Ill.

750. DUMM, M. E., and RALLI E. P *Effects of diet, partial hepatectomy and growth promoting factors on the composition of the rat liver* Federation Proc. 11 440-441 March 1952.

"Adult rats were maintained on a 9% protein, 38% fat diet for 42 days and were then partially hepatectomized. The liver removed was analyzed for lipids, nitrogen and solids. After operation, the rats received the same diet without any supplement or with injections of liver extract or vitamin B_{12}. Other rats were transferred after operation to 16 or 26% protein (9% fat) diets. The animals were sacrificed 6-15 days after operation and the analyses repeated. In the rats continued on the 9% protein diet with no supplement liver lipids increased and nitrogen decreased from operation to sacrifice. Injections of liver extract or increasing the protein in the diet had some lipotropic effect and also increased liver nitrogen. Vitamin B_{12} had a pronounced and consistent lipotropic effect associated with an increase in liver nitrogen. Microscopically the livers of rats on the low protein diet with no supplement showed increased lipid deposition at sacrifice. Rats given liver extract or the higher protein diets showed decreased liver fat and evidences of cellular regeneration. Rats receiving vitamin B_{12} showed striking evidences of cellular regeneration."

New York University-Bellevue Medical Center
New York, N Y

Lipotropism

751 HALL, C. A., and DRILL, V A. *Lipotropic effect of liver extract on dietary hepatic injury in rats*, Proc. Soc. Exper Biol. & Med. 69 3, Oct. 1948.

Fatty change and fibrosis occurring in the livers of rats fed on a diet high in fat but not low in protein were prevented by supplementing the diet with choline or crude liver extract. The lipotropic effect of the liver extract is not due to an increase in protein intake, nor to the small amount of choline present in the liver extract.

752. RODNEY G., SWENDSEID M. E., and SWANSON L. *The role of pteroylglutamic acid in tyrosine oxidation by rat liver tissue* J Biol. Chem. 179 19-24 May 1949

Liver tissue from rats with a succinylsulfathiazole-induced deficiency in pteroylglutamic acid (PGA) shows a decreased oxidation of tyrosine compared with normal liver tissue. The oxidation of tyrosine can be partially restored by the addition in vitro of PGA, but not by PGA conjugate or by liver extract. Liver tissue from rats with PGA deficiency induced by feeding 4-amino-PGA also shows decreased tyrosine oxidation In vitro this effect cannot be reversed by the addition of PGA, liver extract, or vitamin B_{12} concentrate but in vivo it can be reversed by the administration of either PGA or liver extract. The addition of 4-amino-PGA in vitro to normal rat liver does not affect the rate of tyrosine oxidation.

Parke, Davis and Company
Detroit, Mich.

753. DRILL, V A., and McCORMICK, H. M. *Lipotropic effects of vitamin B_{12} concentrate* Proc. Soc. Exper Biol. & Med. 72 388-390, Nov 1949

Injection of vitamin B_{12} concentrate into rats on a high-fat diet exerted a marked lipotropic effect which was not due to the small amount of choline present in the concentrate.

754 SCHAEFER, A. E., SALMON W D., and STRENGTH, D R.: *Role of vitamin B_{12} and methyl donors in lipotropism and transmethylation in the rat and chick*, Federation Proc. 9 369-370, March 1950.

"The relation of vitamin B_{12} to the quantitative effectiveness of monomethylaminoethanol, dimethylaminoethanol, betaine, DL-methionine and choline as methyl donors for the prevention of renal damage and fatty livers in rats and for growth and perosis-prevention in chicks was studied. The choline requirement of weanling rats for protection against fatty livers was reduced from 0.16% to 0.10% when the diet was supplemented with vitamin B_{12} and folacin. On the basis of methyl content monomethylaminoethanol and betaine were considerably less active for growth and prevention of renal damage and fatty livers in the rat than dimethylaminoethanol or choline when vitamin B_{12} and folacin were omitted from the diet. In the presence of vitamin B_{12} all the above compounds were equivalent to choline on a methyl basis. Three mols of betaine were required to replace 1 mol of choline. For the chick, betaine, DL-methionine or monomethylaminoethanol, even in the presence of vitamin B_{12}, could not replace choline for growth and protection against perosis. Monomethylaminoethanol and either DL methionine or betaine could replace choline on a methyl basis only when supplemented with vitamin B_{12}. Vitamin B_{12} appears to function in methylation processes."

Alabama Polytechnic Institute
Auburn, Ala.

755 McCORMICK, H. M., and DRILL, V A.: *Lipotropic effects of liver extract, vitamin B , and choline*, Proc. Soc. Exper Biol. & Med. 74 626-630 July 1950.

In experiments with rats on a high fat diet, it was found that 1 cc. of crude liver extract given subcutaneously three times a week was approximately the minimum effective lipotropic dose. Given orally this amount had slightly less lipotropic effect, and lower subcutaneous doses were less effective.

Since both the liver extract and the vitamin B_{12} concentrate previously reported to have lipotropic action (Drill and McCormick, *Proc. Soc. Exper Biol. & Med* 72 388, 1949 [Abstr 753]) contain small amounts of choline, folic acid, and inositol, a combination of these substances was tested, but was found to be ineffective

Crystalline B_{12}, even in doses of 2.5 mcg. six times a week subcutaneously was ineffective, and combinations of B_{12} and choline did not prevent fatty changes in the liver although animals receiving this combination gained more weight than animals receiving choline alone.

The authors conclude that the lipotropic activity of liver extract or of vitamin B_{12} concentrate must be due to factors other than those discussed above. The possible effects of combinations of crystalline vitamin B_{12} and suboptimal amounts of folic acid, with or without choline, are being studied.

Wayne University College of Medicine
Detroit, Mich.

756. KELLEY B., TOTTER, J R., and DAY P L.: *The lipotropic effect of folic acid on rats receiving various purified diets*, J Biol. Chem. 187 529-535, Dec. 1950.

The addition of vitamin B_{12} to the diets used in these experiments brought about little alteration in any of the fat fractions in the liver Since the diets used contained 18 per cent casein, it is probable that the requisite amount of vitamin B_{12} was thus supplied, so that additional amounts of this vitamin might be expected to have no appreciable effect.

University of Arkansas
Little Rock, Ark.

757 LUCAS, C. C., RIDOUT J H., PATTERSON J M., and BEST C. H. *Lipotropic properties of vitamin B_{12}*, Federation Proc. 10 218, March 1951.

"The lipotropic properties of vitamin B_{12} have been assessed in weanling male rats of the Wistar strain. Effects upon rate of growth, deposition of liver lipids and development of renal lesions have been followed for periods up to 17 days. The interrelationships among choline chloride, vitamin B_{12} and folic acid have been examined by supplementing a hypolipotropic basal diet with each one separately at several levels, and in various combinations. Effects of the vitamins on food intake were controlled by conducting both *ad libitum* and paired feeding experiments. Vitamin B_{12} had no effect on the liver lipids in these experiments."

University of Toronto
Toronto, Ontario, Canada

758. STRENGTH, D R., SCHAEFER, E. A., and SALMON W D.: *The relation of vitamin B_{12} and folacin to the utilization of choline and its precursors for lipotropism and renal protection in rats* J Nutrition 45 329-343, Nov 10, 1951

Vitamin B_{12} and folacin were ineffective as lipotropic substances when fed singly to rats receiving a fied basal diet supplemented with 0.1 per cent ch

chloride. A combination of B_{12} and folacin reduced liver fat from an average of 43 to 18 per cent. Liver fat of the rats fed the basal ration fortified with 0.08 and 0.12% choline chloride and also with B_{12} concentrate and folacin was equal to that of rats receiving 0.12 and 0.16% choline chloride respectively without supplements of B_{12} and folacin. Choline chloride at an 0.20% level was approximately as effective for the maintenance of normal liver fat as was the same level of choline chloride plus B_{12} and folacin. The lipotropic activity of vitamin B_{12} and folacin appeared to consist of a choline-sparing effect, which was manifest at subprotective levels of choline. Vitamin B_{12} and folacin added to the basal ration supplemented with varying levels of betaine or dl-methionine exerted a marked sparing effect on the requirement of these substances for maximum growth, prevention of renal damage, and lipotropism.

The addition of betaine HCl or dl-methionine at an 0.08% choline chloride equivalent level, without B_{12} and folacin resulted in a 100% incidence of renal damage as compared to 13% for choline chloride. Three mols of betaine HCl or dl-methionine, when added to the diet supplemented with B_{12} and folacin were as effective as one mol of choline for protection against renal damage and lipotropism.

Neither dimethylaminoethanol nor methylaminoethanol fed to rats at varying levels was equal to choline for growth promotion and maintenance of normal liver fat. Increasing the level of methylaminoethanol or dimethylaminoethanol in the absence of B_{12} and folacin resulted in a depression of body weight gain. When these compounds were fed at a choline chloride equivalent level of 0.16% (without B_{12} and folacin) average liver fat values were 36 and 26% respectively as compared with 17% when choline was fed. The addition of B_{12} and folacin reduced liver fat. Dimethylaminoethanol was considerably more active than methylaminoethanol. Trimethylcholine was not capable of replacing choline as a lipotropic agent for weanling rats.

With a more highly purified diet, in which the casein and peanut meal were thoroughly extracted with methanol, supplementation with betaine or dl-methionine at a choline equivalent level of 0.08% plus vitamin B_{12} and folacin, resulted in 100 and 30% renal damage, respectively. When vitamin B_{12} and folacin were added to the highly purified diet supplemented with methylaminoethanol and dl-methionine at a choline equivalence of 0.12% the lipotropic activity of this combination was equal to that of choline. When B_{12} and folacin were not included, the lipotropic activity was not equal to that of choline. The addition of dl-methionine, or betaine HCl, or dl-methionine plus aminoethanol at a choline chloride equivalent level of 0.08% resulted in 100% renal damage in rats on the more purified diet. Supplementation of this ration with B_{12} and folacin, and either dl-methionine or betaine HCl, resulted in 30 and 100% renal damage, respectively. Further addition of aminoethanol to these diets gave complete protection against renal damage and supported maximum growth.

Results obtained in these experiments indicate that B_{12} and folacin appear to be essential for the maximum utilization of methionine or betaine for the biological synthesis of choline from aminoethanol or methylaminoethanol.

Alabama Polytechnic Institute
Auburn, Ala.

759 FISCHER, M. A., and HALL, G. D.: *Relation of dietary protein to signs of choline deficiency—effect of supplements of vitamin B_{12} and folic acid*, Federation Proc. 11 211-212, March 1952.

"By the tenth day 75% of the rats fed a diet containing 15% casein showed signs of acute choline deficiency. The kidneys were enlarged and hemorrhagic, the serum non-protein nitrogen had risen to 70 mg.% and the livers contained an excess quantity of fat. Addition of B_{12} and folic acid to this diet reduced liver fat and prevented kidney damage. Both control and supplemented groups fed a 9% casein ration exhibited poor growth and neither group showed signs of choline deficiency however the lipotropic effect of the supplement was evident. When animals were fed a 24% casein diet the vitamin supplements had no apparent effect on growth response, liver weight and fat content, or kidney weight. The serum NPN of both of these groups was about 30 mg.%. In contrast, the supplement reduced the NPN from 26 mg.% to 20 mg.% when the animals were maintained on 9% casein. Low values were also observed when the supplemented 15% casein diet was fed. Serum albumin content was low in animals fed the 9% casein diet. B_{12} and folic acid additions to the diet slightly raised the level. The supplements had least effect on serum albumin of rats fed the 24% casein diet. Changing the protein content or adding the vitamins to the diets caused some changes in the serum globulins."

University of Pittsburgh School of Medicine
Pittsburgh, Pa.

760. BEST, C. H., HARTROFT, W. S., and SELLERS, E. A.: *The protection of the liver by dietary factor* Gastroenterology 20 375-384, March 1952.

This general descriptive (primarily histologic) article summarizes present knowledge on the effects of choline deficiency on the liver of experimental animals. Choline deficiency causes an intensely fatty liver and loss of liver function. Restoration of choline to the diet cures these conditions. Cirrhosis due to choline deficiency in animals can be cured by choline if the cirrhosis is not too advanced. The signs of choline deficiency are apparent within 24 hours of the beginning of the low-choline regimen and within a week all the liver cells are distended with fat. Stainable lipid first appears in the liver as small intracellular globules which coalesce to form large masses which distend the cell membrane and then rupture it so that fatty cysts are formed. Later these involve biliary and vascular channels. The stages of removal of fat from the liver upon restoration of choline to the diet are described and illustrated in detail. Intracellular fat disappears more rapidly than extracellular fat, the latter taking several months.

It is pointed out that caloric restriction has a "pseudo-lipotropic" effect. Clinicians and physiologists have tried to cure by choline administration many types of liver damage which were not caused by choline deficiency. This, in the authors' opinion is futile.

Commenting on the lipotropic effects of vitamin B_{12} and folic acid, the authors state "Folic acid and vitamin B_{12} are now involved in lipotropic phenomena and the amounts of these substances present in a diet may prove to affect liver function through mechanisms not directly related to those which control anemia. Methyl groups (CH_3) are important in the supply of lipotropic agents since their addition to ethanolamine, which is usually available in the body may form choline. These vitamins, folic acid and B_{12}, by favoring the synthesis of new methyl groups, may affect the apparent choline requirement of an animal and perhaps of a patient." Present attempts to use lipotropic agents therapeutically are not based on the known action of these substances in humans, but on their effects in experimental animals. If some means could be found "to produce a safe but definite choline deficiency in human subjects, the most important single step toward integrating laboratory and clinical findings in this field would have been taken."

University of Toronto
Toronto, Canada

761. TRAVERS, J J., and CERECEDO L. R.: *Interrelation between choline and vitamin B_{12} in the mouse*, Federation Proc. 10 396-397 March 1951

"The greater resistance of the mouse to choline deficiency and the involvement of vitamin B_{12} in the synthesis of methyl groups and choline led to a study (microbiological) of the vitamin B_{12} content of mouse and rat tissues. Under all conditions mouse liver contained more vitamin B_{12} than rat liver In each species lowest values were obtained with a sulfasuxidine-iodinated casein diet which, after 5 weeks, produced values for mouse liver comparable with those of stock rats, and a 6-fold increase over those of rats similarly treated. A vegetable protein diet caused a temporary decrease in each species, followed by an increase. Even after 8 months, mouse liver contained as much vitamin B_{12} as those of young stock mice, and more than those of young stock rats. Thus, as with choline, younger animals need an exogenous supply of vitamin B_{12}. Since this vitamin is involved in the synthesis of methyl groups and the transmethylation process, it appears that the resistance of the mouse to choline deficiency can be related, in part, to its vitamin B_{12} content. The data are also in line with our inability to produce in mice, in contrast to what has been observed in rats, tumors by choline deficiency with the recent report on the procarcinogenic effect of vitamin B_{12} in rats and with the greater incidence of spontaneous tumors in mice."

Fordham University
New York, N Y

762. TRAVERS, J J., and CERECEDO L. R. *Interrelationships between choline and vitamin B , in the rat and in the mouse*, Federation Proc. 11 457 March 1952.

"Rapid and uniform depletion of the vitamin B_{12} stores of the livers of rats was accomplished with a diet containing sulfasuxidine and iodinated casein, but free of vitamin B_{12} and choline. The growth of these rats was stimulated by the administration of vitamin B_{12}, and of choline. Vitamin B_{12} was found to be without effect if choline was present in the diet at an adequate level. Under the conditions used, choline, supplied in the diet, prevented to a large extent the depletion of the liver stores of vitamin B_{12}. The choline content of the kidneys of choline deficient rats was significantly lower than that of choline fed rats, and was not affected by the administration of vitamin B_{12}. Under similar conditions, different results were obtained with mice. The administration of choline had no effect on the vitamin B_{12} content of the livers of mice, nor on the choline content of the kidneys of mice. "

Fordham University
New York, N Y

763. SCHAEFER, A. E., COPELAND D H., and SALMON W D *Duodenal ulcers, liver damage anemia and edema of chronic choline deficiency in dogs*, J Nutrition 43 201 222, Feb. 1951

The effects of choline deficiency were studied in dogs. Supplementing the diet of the dogs with 0.05 per cent or less of choline resulted in death in 13 to 30 weeks. The symptoms of choline deficiency preceding death were anemia, liver dysfunction, weight loss, and edema. Hepatic function became impaired in chronically choline-deficient dogs four to eight weeks before anemia developed. Subcutaneous edema and excessive fluid in the abdominal cavity were noted in 6 of the 7 dogs that died. Vitamin B_{12}, which has a sparing action on the choline requirement of rats and chicks, also protected dogs against the effects of choline deficiency in the present experiments. A vitamin B_{12} concentrate was used. The inclusion of a source of vitamin B_{12} in the diet prevented the development of ascites. In dogs not receiving vitamin B_{12}, the choline requirement was 0.10 per cent or greater Autopsy studies revealed that 6 of 7 dogs who died of chronic choline deficiency had duodenal ulcers (perforated in 2) Whether these ulcers were specifically related to choline deficiency or were a secondary result of the accompanying inanition could not be determined. The livers of all choline-deficient dogs were fatty and 6 dogs had cirrhosis.

Alabama Polytechnic Institute
Auburn, Ala.

764 BURNS, M. M., and McKIBBIN J M. *The lipotropic effect of vitamin B_{12} in the dog*, J Nutrition 44 487-499 Aug. 1951.

Authors summary "1. A deficiency of vitamin B_{12} is reported in weanling puppies fed on a synthetic diet containing 19% purified casein and 9% fat. The deficiency varied in severity with different litters of animals and was evident in growth impairment, abnormalities of liver function and, in several instances, gross fatty infiltration of the liver

"2. Administration of either vitamin B_{12} or choline produced significant improvement in liver function in 4 dogs receiving the basal diet. Two dogs receiving a choline-deficient diet were cured of their fatty livers with Vitamin B_{12} alone.

"3. These findings suggest that the dog s requirement for methylating substances, like that of the rat and ch k is dependent upon the vitamin B_{12} content of

Syracuse University
Syracuse, N Y

Radiation Sickness, Injury

765. WARREN, S. *Atomic Energy Commission report,* J Indiana M. A. 42 1082-1084, Oct. 1949

In a report of the Atomic Energy Commission to Congress the importance of the bone marrow and the blood in radiation sickness is mentioned. Repeated blood transfusions are needed by people exposed to atomic radiation, and it is suggested that new substances, such as vitamin B_{12}, which stimulate the formation of mature blood cells, will be helpful in radiation sickness.

766. PARK, W E. *If an atomic bomb should fall,* Canad. M. A. J 61 473-478, Nov 1949

The treatment outlined for victims includes prevention of infections by administration of penicillin in daily doses of 200,000 to 1,000 000 units, preferably by mouth streptomycin if the patient develops septicemia of Escherichia coli infection daily administration of liver extract or vitamin B_{12} and folic acid to promote the formation of red blood cells.

Chalk River Ontario, Canada

767 REZNIKOFF P., in Cornell Conferences on Therapy: *Problems in treatment of the atomic casualty* New York State J Med. 52: 1804, July 15, 1952.

In a discussion on this subject the question of the effect of antibiotics, particularly aureomycin, on radiation sickness was raised. Dr Reznikoff cited two studies that contribute to understanding of the mechanism of action of aureomycin. Dr Davis has shown that Bacterium coli organisms can absorb or inactivate large amounts of vitamin B_{12}. Watson and Jukes have obtained good results in pernicious anemia from the combined administration of B_{12} and aureomycin. Since B_{12} is not ordinarily effective when given orally in this disease, it is presumed that aureomycin indirectly protected the vitamin and thus permitted it to be utilized. A similar situation may occur in radiation sickness. Aureomycin may by means of its antibiotic activity on the intestine, permit absorption of essential therapeutic substances.

Cornell Medical College, and New York Hospital New York, N Y

768. BUTLER, R. E.: *Special feeding problems in an emergency* Pub. Health Rep. 67 867-871 Sept. 1952.

Among the subjects discussed is the intravenous and oral use of fat emulsions in feeding burned patients. As an oral preparation, the fat emulsions have also been investigated for their effect in increasing caloric intake. Recommendations are also given for the treatment of shock in an emergency

In the discussion of radiation injuries the author states that there is little indication that such hemopoietic agents as folic acid and vitamin B_{12} would be effective, and that iron would seem to be useless until blood regeneration is observed.

Office of the Surgeon General U. S. Public Health Service Washington, D. C.

769 CORNATZER, W E., ARTOM, C., HAI T., and CAYER, D *Beneficial effects B₁₂ and folic acid on recovery from inte tion by P³²* Proc. Soc. Exper Biol. & 552-554, March 1951.

Generous amounts of folic acid, or folic vitamin B_{12} had no significant effect on the mice injected with a dose of P^{32} in the highe LD_{50}. When sulfasuxidine was included in the ever the administration of vitamin B_{12} and increased significantly both survival time and of survivors.

Bowman Gray School of Medicine Wake Forest College Winston-Salem, N C.

Antithyrotoxic Effects

770. NUTRITION NOTES: *The anti-thyrotoxic vitamin B_{12}*, Am. J Digest. Dis. 16 418,

An antithyrotoxic effect of vitamin B_{12} fore the possible usefulness of this vitamin in ment of hyperthyroidism are suggested by 1) that individuals with pernicious anemia comp severe hypothyroidism require a much high of thyroid extract when they are placed on live 2) the finding of Ershoff that feeding liver co rotoxicity in rats, and 3) the observation th amounts of vitamin B_{12} duplicate the growth of fish solubles or injected liver extracts in a ceiving thyrotoxin.

771 NUTRITION NOTES *The anti-thyrotoxic vitamin B_{12}*, Am. J Digest. Dis. 17: 25,

It has been shown that vitamin B_{12} has a rotoxic effect. For example, patients suffering nicious anemia complicated by severe hypot may require a higher dosage of thyroid extract cases, double the amount) than they needed pr therapy Several other examples are cited. It is that B_{12} may eventually be useful in the tre hyperthyroidism. Practitioners are cautioned administration of thyroid extract is less effect tients who are receiving either liver extrac therapy

772. WAYNE, E. J., MACGREGOR, A. G., and H. *Vitamin B , and thyroid function,* 827 Feb. 18, 1950 (in Letters to the Edi

On the basis of tests with two male pati normal thyroid function, it is concluded that in of 100 mcg. or less, vitamin B_{12} has no signific on thyroid function.

University of Sheffield, and Sheffield National Centre for Radiotherapy Sheffield, England

773 QUESTIONS AND ANSWERS: Mod. Med. 20 1 1952.

"QUESTION Does 50 µg. of vitamin B_{12} given intra muscularly have any effect on the basal metabolic rate? Does this vitamin occasionally cause a rise in temperature?

"ANSWER: By Consultant in Pharmacology Vitamin B_{12} has no effect on the basal metabolic rate. An increase in body temperature at the time of injection is probably caused by the presence of undesirable pyrogens."

ANEMIAS OF TOXICITY; OTHER TOXIC STATES

774 VON BONSDORFF B.: *Étude sur la vitamine B_{12} (Study regarding vitamin B_{12})* Semaine d. hôp. de Paris 26: 3316-3317 Sept. 2, 1950.

Treatment of pernicious anemia due to the fish tapeworm (Diphyllobothrium latum) consists of expulsion of the tapeworm, or of administration of liver gastric preparations, or folic acid orally or parenteral administration of liver folic acid or vitamin B_{12}. Patients with this disease have reacted in two ways to vitamin B_{12} administered orally 5 mcg during eight days. Some of these patients have shown no response to vitamin B_{12} either with or without gastric juice, while in others remission is obtained even without the administration of gastric juice. Secretion of the intrinsic factor in sufficient quantity would evidently establish vitamin B_{12} action, and absorption of this product at a high level in the digestive tract where the parasite could not endure the reaction, would follow

Helsinki, Finland

775. FRANK, O., LACHNIT V., and NEUMAYR, A.: *Influence of vitamin B_{12} on lead porphyrinuria*, Acta haematol. 8 42- 1952 (abstr J.A.M.A. 150 1433, Dec. 6, 1952)

"Increased urinary coproporphyrin is a constant and early sign of lead poisoning. This sign is important in the diagnosis but also presents a therapeutic problem, since it is said to be related to a number of symptoms, particularly colic and neuritic symptoms. The favorable effects produced with liver extracts and the components of the vitamin B complex induced the authors of this report to try vitamin B_{12}. They present observations on 11 patients whose occupations involved exposure to lead. They were given 30 µg. of vitamin B_{12} twice weekly by intramuscular injection. The elimination of coproporphyrin in the urine was determined at weekly intervals. Treatment with vitamin B_{12} normalizes porphyrinuria and prevents the excessive urinary excretion of coproporphyrin induced by mobilization of lead. This effect of vitamin B_{12} is believed to be the result of its catalytic action on hemoglobin synthesis, especially on the ribonucleotides and of its protective action on the liver"

776. TUCZEK, H., and SAUPE, M. *Experiences with isonicotinic acid hydrazide in tuberculosis* Munchen. med. Wchnschr 94 1307 June 27 1952 (abstr J.A.M.A. 150 1052, Nov 8, 1952)

This report describes the studies in a sanatorium in which more than 1,000 tuberculosis patients were under treatment for 8 to 12 weeks. In discussing effects on various systems, it was noted that in 84 patients the sedimentation rate remained unchanged, while in 108 others, it became normal. "About 30% of the patients showed a decrease in erythrocytes after treatment with isonicotinic acid hydrazide had been continued for some time, but the erythrocyte count became normal following treatment with vitamin B_{12}."

777 KRESBACH, E., and KNORR, G.: *Panmyelophthisis after treatment with methylphenylethylhydantoin*, Klin. med. 7: 346- Aug 1 1952 (abstr J.A.M.A. 150 1349 Nov 29 1952)

"The patient was a young woman, aged 19 who had had epileptic attacks since the age of 14. For some time she had been treated with phenobarbital, but then the medication had been changed to methylphenylethyl hydantoin. After many months of this treatment she had become pale, and severe anemia had been detected for which several blood transfusions had been given. At the present time the results of sternal puncture and of blood examinations indicated severe panmyelophthisis. In addition to blood transfusions and hemostyptic drugs, large doses of vitamins C, B_2 and K were given, and attempts were made to stimulate hematopoiesis by vitamin B_6 and B_{12}, folic acid, and large doses of iron. Under the influence of these measures, the cutaneous and mucosal hemorrhages were temporarily arrested, but the septic fever remained unchanged. Later mucosal hemorrhages recurred, coma developed, and the patient died. An autopsy was performed, and it was assumed that the hydantoin preparation has produced toxic destruction of the bone marrow"

778. STEIM, H., and WEISSBECKER, L. *Symptomatic pernicious anemia in chronic poisoning with chromium*, Zentralbl. Arbeitsmed. u. Arbeitsschutz, Darmstadt 3 42 March 1953 (abstr J.A.M.A. 152 1575, Aug. 15, 1953)

"The occurrence of hyperchromic anemia leukopenia, reduced number of reticulocytes (0.2%) Hunter's glossitis, histamine-refractory gastric anacidity and paresthesia in a workman is reported who had been employed for 25 years in a tannery The patient had cachexia and islet-like surface defects in the oral cavity and the esophagus, which, in the presence of large amounts of chromium in the saliva and in the urine, completed the characteristic picture of chronic chromium poisoning The classic symptoms of pernicious anemia prompted the author to give the patient vitamin B_{12} and/or pteroylglutamic (folic) acid orally or parenterally but the patient did not respond to this treatment. He then was given continued blood transfusions. Six months after the last blood transfusion the blood counts had become normal. The ulcers in the mouth and in the esophagus were cicatrized and the amounts of chromium in the urine and in the saliva were considerably reduced, but the cachexia and the atrophy of the lingual papillae remained unchanged. There are two possible explanations for the failure of vitamin B_{12} therapy in this patient. The synthesis of the thymonucleic acid from thymidine may have been disturbed directly by the chromium, or vitamin B_{12} may have been inactivated by the chromium as a result of its complex affinity"

779 MAURIZIO E. *Vitamin B_{12} in medical therapy of toxemia of pregnancy and eclampsia*, Minerva Ginecologica 4 553 Nov 1952.

780. MAURIZIO E. *Vitamin B_{12} (cyanocobalamine) in the medical therapy of toxemias of pregnancy and eclampsia*, Minerva med. 1 571-572, March 7 1953.

The author states that in patients with toxemia of pregnancy and eclampsia medical management is designed to combat the three derangements always present in each variety of the eclamptic syndrome, namely convulsions, angiospasm and toxicity He points out that as a result of widespread alterations in the general metabolism of the body the toxic state involves the entire organism. The mechanism of detoxification is accomplished by means of a large mass of oxidative, reductive and conjugative reactions which take place principally in the liver and kidneys. Detoxification therefore rests, to a large extent, upon the functional integrity of these organs.

The author s "detoxification" regimen in these patients is discussed in considerable detail. Mention is made of his experiences, dating from 1946, with the use of nicotinic acid and liver extracts in the treatment of the "toxicity state" of these patients. The author avers that his results with these agents over a period of four years were good. He explains that the actions expected from the use of nicotinic acid were two (a) a hypotensive effect (b) a "hepato-protective" effect. The presupposition upon which his use of nicotinic acid was made in these patients is based upon the thought that this chemical, which has coenzymic activity participates in the formation of an important enzymatic system which intervenes in respiratory-cellular processes, that is codehydrogenase I and II, which activate redox processes of the liver cell. (In general, the doses of nicotinic acid employed varied from 50 to 150 mg daily; rarely were doses of 200 mg. daily given.)

Impressed by the clinical efficacy of liver extracts in the eclamptic syndrome, the author began to use vitamin B_{12} in an effort to obtain hepatoprotective and detoxification effects in these patients. Recent researches, evaluated by the author in which B_{12} was employed in the treatment of patients with the eclamptic syndrome, revealed that the vitamin has biologic activity which the author summarizes as follows 1. Stimulates the synthesis of nucleic acids. 2. Favors transmethylation. 3. Develops lipotropic activity In an attempt to elucidate the mechanism of the action of B_{12}, the author found it to possess a pronounced "hepatotropic" and "nephrotropic" effect, stimulating a remarkable increase in the activity of "betaglucuronidase" and an "aspecific cholinesterase" of the liver and kidneys.

Based upon these observations the author treated "numerous patients" with "toxicity of pregnancy" with doses of B_{12} which varied from 20 to 50 mcg. daily more frequently doses over 30 mcg were used. "Consistently satisfactory" results were obtained as evidenced by a notable improvement in the patient's general condition and in liver and kidney function. The efficacy of this therapy was observed within 24 to 48 hours. In none of the patients treated were eclamptic convulsions seen. "An increased diuresis was observed in all patients treated."

The University of Genoa
Genoa, Italy

ANIMAL STUDIES

Physiologic Agents

781 TRAINA, V *Vitamin B_{12} and histamine*, Nature 165 439-440, March 18, 1950 (in Letters to the Editors)

Four guinea pigs, serving as controls, were given intracardiac injection of 0.12 mg. of histamine (almost twice the MLD by intravenous injection) Within half an hour all of them had died of shock. Eight more guinea pigs were given 15 mcg. of vitamin B_{12} intraperitoneally later each received a 1-mg. intracardiac injection of histamine. None of them died, although very slight signs of shock were noted. It is the writer's impression that the same dose of B_{12} was more effective in histamine shock than in anaphylactic shock in the guinea pig. From a study of the contraction response of guinea pig intestine in vitro, the writer concluded that vitamin B_{12} does not act by direct inactivation of histamine. In another experiment on guinea pigs the writer obtained results which indicate that the B_{12} does not activate histaminase. On the basis of the experiments cited, it is concluded that the mechanism of action of B_{12} seems to be competitive antagonism as in the case of the common antihistaminic drugs.

Breno, N Y

782. TRAINA, V *Vitamin B_{12} as an anti-anaphylactic*, Nature 166 78-79 July 8 1950.

Following the hypothesis that macrocytic anemia could be due to an anaphylactic blockage of the bone marrow experiments were conducted which showed that vitamin B_{12} had a protective action against anaphylactic shock in sensitized guinea pigs when it was administered 15 minutes prior to a shocking dose of horse serum. It was effective in doses as low as 60 to 90 mcg. per Kg. of body weight. This dose is one-thirtieth to one-fiftieth as large as that required when antihistaminic agents are used to protect guinea pigs from anaphylactic shock.

Fairview Park Hospital
Cleveland, Ohio

783 SHARPE, H. M., WOOLLETT E. A., and CUTHBERTSON W F J *Vitamin B_{12} and antihistaminic activity* Nature 166 651-652, Oct. 14 1950 (Letter to Editors)

Subcutaneous injection of 50 to 500 mcg. of vitamin B_{12} did not protect guinea pigs from having a fall in blood pressure after intravenous doses of histamine. Subcutaneous injection of 15 mcg. of vitamin B_{12} per Kg. of body weight did not protect these animals from bronchospasm, often fatal, when histamine was injected intracardially

Glaxo Laboratories Ltd.
Greenford, Middlesex, England

784 DUCROT R. *Vitamin B , and histamine* Compt. rend. Soc. de biol. 144 697 1950 (Allergy Abstr 16 94, in J Allergy 22 Nov 1951)

"Vitamin B_{12}, which is said to have an antihistaminic action, failed to exert any protective effect against histamine shock induced in guinea pigs by the inhalation of aerosolized histamine or by the intravenous injection of histamine. Vitamin B_{12} also failed to show antihistaminic properties in Dale experiments with isolated guinea pig intestine."

785. NARANJO P: *Histamine shock and vitamins* Proc. Soc. Exper Biol. & Med. 81 111 113, Oct. 1952.

Author s summary: "1 Even in high doses, vit. B_1 B_{12}, C, K, and B complex did not protect guinea pigs against histamine shock produced by inhalatory administration of histamine aerosol. 2. Vit. B_{12} and C did not exhibit any antihistaminic action in assays of blood pressure in cats and guinea pigs. 3. Vit. B_{12} and C did not show any antihistaminic action in assays on the isolated guinea pig ileum in high doses both vitamins caused the intestinal strip to relax, but without decrease of its histamine sensitivity"

Central University of Ecuador
Quito, Ecuador

786. LUDOVICI, P P., and AXELROD A. E. *Circulating antibodies in vitamin-deficiency states. Pteroylglutamic acid niacin-tryptophan, vitamins B_{12} A and D deficiencies*, Proc. Soc. Exper Biol. & Med. 77 526-530, July 1951

Authors summary "1 Hemaglutinin production in response to inoculation with human erythrocytes has been investigated in pteroylglutamic acid, niacin-tryptophan, vit. B_{12}, vit. A, vit. D–deficient rats and their respective controls. 2. Severe impairment of antibody response was observed in the pteroylglutamic acid–deficient rats. Moderate impairment was demonstrated in vit. A and niacin-tryptophan deficiencies. Vit. B_{12} and vit. D deficiencies had no effect on antibody formation. 3 A rough classification of the effect of 10 deficiency states upon antibody production is presented."

University of Pittsburgh
Pittsburgh, Pa.

787 AXELROD A. E.: *Role of the vitamins in antibody production (A summation of the author s work to date)* Metabolism 2 1-8, Jan. 1953.

Author s summary "The available evidence would indicate that certain B vitamins, notably pyridoxine, pantothenic acid and pteroylglutamic acid, play a significant role in antibody synthesis. There is considerable basis for linking pyridoxine to amino acid metabolism, and its participation in antibody production may be related to this function. A corresponding mechanism for the action of pantothenic acid has not yet been demonstrated. A relationship between pantothenic acid and peptide bond formation is suggested by the observation of Chantrenne that coenzyme A, a compound that contains pantothenic acid, is involved in the synthesis of hippuric acid.

"The ability to control antibody production by regulating the vitamin intake should be a useful tool in the unraveling of the mechanisms of antibody synthesis. Since the antibodies represent a class of specialized proteins, such studies would yield information regarding the processes of protein anabolism in general. The fact that the measurement of antibodies is greatly facilitated by the application of specific immunological procedures renders them particularly suitable for studies of protein synthesis."

In the experiments referred to, in which mice were used, neither vitamin B_{12} nor vitamin D deficiency states had any effect on antibody formation. There was moderate impairment of antibody formation in mice which were deficient in riboflavin, thiamine, biotin, vitamin A and niacin-tryptophane.

Institute of Pathology and
Western Reserve University
Cleveland, Ohio

788. OGASAWARA, K., ATA, S., HISADA, S. *Vitamin B_{12} and acetylcholine shock* Lancet 1 598-599 March 21 1953 (in Letters to the Editor)

In experiments with mice weighing 12 Gm., it was found that 15 mcg. of vitamin B_{12} given intraperitoneally 15 to 90 minutes before an MLD (minimum lethal dose) of histamine given intravenously was sufficient to protect all mice from death and from most signs of shock. When the vitamin was given 120 minutes before the histamine there was no protection from shock and death.

Mice kept for a week on a deficient diet showed less resistance to histamine (MLD was halved) but 15 mcg. of vitamin B_{12} intraperitoneally protected them from 1 7 MLD of histamine given 15 minutes later

In the doses given (15 and 30 mcg.) vitamin B_{12} intraperitoneally did not protect the mice from shock and death from acetylcholine (15 mg. per kg or MLD) However when 12 mcg. of vitamin B_{12} was given intravenously at the same time as the acetylcholine, all mice survived. All died when the amount of vitamin was decreased or acetylcholine increased.

In rabbits it was found that 5 to 30 mcg. of vitamin B_{12} per Kg. given 5 minutes before 5 mg. of acetylcholine per Kg. did not modify the effect of acetylcholine on blood pressure.

Nagoya University School of Medicine
Japan

789 MEYER, L. K., and DAVIS, J E. *Anemia produced by acetylcholine in guinea pigs*, Federation Proc. 12 350 March 1953.

"Eight normal male guinea pigs which had received a uniform diet of Purina Rabbit Chow were found to have red blood cell counts of approximately 6 million. After determining the correct resting erythrocyte number for each animal, we administered daily subcutaneous injections of acetylcholine bromide (2 mg.) This caused reductions in the erythrocyte numbers of all of the animals. Normal counts were diminished by 29-71% (average 49.6) within 21-67 days. These decreases represent the maximum degrees of anemia which were reached in an average period of 35 days of acetylcholine injections. Three of the animals died at times during which their anemias were most severe. One animal showed a peak reticulocytosis of 21% on the 4th day of therapy with vitamin B_{12} in spite of daily injections of acetylcholine. However neither vitamin B_{12} nor folic acid alone

found to cause complete regeneration of the red blood cells. In a few animals combined treatment with both folic acid and vitamin B_{12} resulted in the restoration of the erythrocyte numbers almost to normal, in spite of continued daily injections of acetylcholine. The cellular type of this experimental anemia was not studied, since our blood samples were obtained by puncturing the small veins of the ears."

University of Texas College of Pharmacy
Austin, Texas

Drug Toxicity

790 BOSSHARDT D K., HUFF J W., PAUL, W J., and BARNES R. H.: *Animal protein factor and growth of mice fed diets containing succinylsulfathiazole,* Federation Proc. 8 378, March 1949

Sharp & Dohme, Inc.
Glen Olden, Pa.

791 JONES, J H., ROGERS, C. S., and STONE, C. H. *Succinylsulfathiazole and a rat growth factor in liver* J Nutrition 39 579-590, Dec. 1949

Young rats grew well on a synthetic diet containing purified casein and all members of the B complex except vitamin B_{12} and folic acid. The addition of succinylsulfathiazole caused failure of growth, leukocytopenia, and then death. The inclusion of folic acid in the succinyl sulfathiazole diet corrected the blood dyscrasia and prevented the death of the animals but failed to restore growth completely Animals given succinylsulfathiazole plus a crude liver extract had normal blood and grew rapidly The subcutaneous injection of a purified antipernicious anemia liver preparation into rats on the diet containing succinylsulfathiazole and folic acid produced very good growth. The authors are unable to conclude definitely that vitamin B_{12} is the factor in liver responsible for the increased growth in the rats receiving succinylsulfathiazole, but believe that this is the case because of the fact that only 0.02 cc. of an antipernicious anemia concentrate produced excellent growth in the rats receiving succinylsulfathiazole and folic acid.

University of Pennsylvania School of Medicine
Philadelphia, Pa.

792. ASENJO C. F: *Influence of vitamin B_{12} on a succinylsulfathiazole-induced dietary deficiency in rats* Federation Proc. 9 147 148, March 1950.

"In the course of investigating the suppletory action of natural products on folic acid-deficient rats receiving a highly purified basal diet containing succinylsulfathiazole, it was noticed that many of the products tested induced a growth response and a leukocyte regeneration at rates well above those obtained with the optimal level of folic acid. This suggested that the depleted animals suffered not only from a severe folic acid deficiency but also, perhaps, from other less severe deficiency or deficiencies. As such a basal diet could have possibly developed a vitamin B_{12} deficiency concurrently with the severe folic acid deficiency it was decided to study the effect that B_{12} supplementation could have in the depleted rats. Preliminary evidence indicates that when high levels of folic acid are given as supplement, the concurrent administration of vitamin B_{12} significantly improved the growth-response and white cell count. At low levels of folic acid supplementation, the addition of B_{12} did not improve the response produced by folic acid alone, though survival was a little longer Folic acid negative controls receiving B_{12} did not show any improvement, but they also lasted longer than the controls without B_{12}. The incidence of infarct like splenic lesions was high in both groups of controls, in those receiving B_{12}, perhaps because they survived longer the splenic lesions developed to such an extent that this organ was found, in several instances, adhered to those adjacent. A level of 0.5 µg. [per] day of B_{12} was used throughout."

School of Tropical Medicine
University of Puerto Rico
San Juan, P. R.

793. FÖLLMER, W *Sulfonamides and fetal development,* Med. Welt 20 58- Jan. 13, 1951 (abstr J.A.M.A. 145 1300, April 21, 1951)

"According to Föllmer sulfonamides administered to a pregnant animal pass the placental barrier and reach the fetus. When the administration is prolonged, the concentration in the fetal serum is about the same as that in the maternal serum. Experiments are described that indicate that prolonged administration of sulfonamides during pregnancy exerts a damaging effect on the fetus, but this harmful effect becomes manifest chiefly after delivery in that growth in length and increase in weight are retarded in the young animals. Apparently compensatory mechanisms in the maternal organism prevent serious damage to the fetus during the intrauterine life, because experiments on tadpoles demonstrated that sulfonamides do inhibit the growth of embryonal tissues. Thus the sulfonamides not only exert bacteriostatic effects but also cytostatic effects on embryonal tissues. The tadpole test, which the author describes, apparently is capable of demonstrating cytostatic effects of various substances, such as vitamin B_{12} and choline, on embryonic tissues."

794 ERSHOFF B. H. *Growth-promoting activity of vitamin B_{12} in mice fed massive doses of atabrine* Arch. Biochem. 26 50-53, March 1950.

Growth retardation in mice due to massive doses of atabrine was only partially counteracted by vitamin B_{12} while extracted liver residue restored growth to normal. This indicates the presence of a factor(s) in liver other than vitamin B_{12} which contributes to growth promotion. It is distinct from any known B vitamin.

795. ATA, S., and TANAKA, K. *Vitamin B_{12} and isoniazid,* Lancet 2 589 Sept. 20, 1952 (in Letters to the Editor)

Experiments with mice are described in which it was shown that large doses of vitamin B_{12} decreased the toxicity of isoniazid. Most effective were doses of 300 mcg./Kg. body weight whether given half an hour before, half an hour after or with a subcutaneous dose of isoniazid. Similar experiments using 30 and 3 mcg. of vitamin B_{12} per Kg. of body weight administered half an hour after the isoniazid had only a slight effect on the LD_{50} of the isoniazid in mice.

Kagoya University School of Medicine
Japan

796. RUBIN B., and BURKE, J C.: *Vitamin B_{12} and isoniazid* Lancet 2 937 Nov 8, 1952 (In Letters to the Editor)

"Recently Ata and Tanaka reported that a subcutaneous injection of vitamin B_{12} (0.30 mg per kg. body weight) appreciably diminished the acute toxicity of isoniazid in mice. In their experiments a definite increase in the subcutaneous LD_{50} dose of isoniazid was obtained when the above-mentioned dose of B_{12} was administered thirty minutes after the isoniazid injection. We have been unable to confirm their results using aqueous solutions of crystalline vitamin B_{12}. Even a 16-fold increase in the dose of B_{12} failed to protect against the acute toxic effects of isoniazid in male albino mice. Isoniazid was administered as a 2% aqueous solution and vitamin B_{12} was injected as a 0.002 or 0.032% aqueous solution. The B_{12} test solutions were checked for qualitative and quantitative content by infra red and ultraviolet absorption measurements. Dose volumes of the B_{12} solutions injected were 0.30 ml. per 20 g. body weight. Isoniazid control mice received equivalent volumes of physiological saline subcutaneously instead of B_{12} solution. The results are shown in the following table:

EFFECT OF VITAMIN B_{12} ON TOXICITY OF ISONIAZID IN ALBINO MICE

Subcutaneous 30 minutes after treatment		Subcutaneous Isoniazid LD_{50} (mg per kg)	No. of Mice
Agent	Dose (mg. per kg.)		
Saline	—	190 (170-213)	30
B_{12}	0.30	196 (180-214)	30
B_{12}	4.80	185 (167-205)	30

19/20 confidence limits indicated in parentheses.

Neither of the two B_{12}-isoniazid LD_{50} doses differed significantly from the saline-isoniazid LD_{50}.

"Among more than fifty compounds screened as possible prophylactics against isoniazid acute toxicity in mice, only barbiturates and some related central depressant agents appeared to offer therapeutically useful protection. Certain thiol compounds, including dimercaprol (BAL) offered only a transient suppressive action in high doses. A large series of other agents, which included carbohydrate metabolites, amino-acids, and vitamins, failed to protect mice unequivocally against the acute toxic effects of isoniazid. These results are to be published in detail elsewhere at a later date."

Squibb Institute for Medical Research
New Brunswick, N J

PRODUCTS OF BODY METABOLISM

797 MENGE, H., and COMBS G F: *Action of vitamin B_{12} in counteracting glycine toxicity in the chick,* Proc. Soc. Exper Biol. & Med. 75 139-142, Oct. 1950.

The results indicate that vitamin B_{12} functions in the metabolism of glycine.

798. STERN J R., and McGINNIS J *Toxicity of glycine for vitamin B_{12}-deficient chicks,* Proc. Soc. Exper Biol. & Med. 76: 233-234, Feb. 1951

Previous reports have indicated that vitamin B_{12} is concerned with nitrogen utilization and metabolism. This was investigated by feeding chicks which had received vitamin B_{12} and vitamin B_{12}-deficient chicks a known amount of nonprotein nitrogen (1 Gm. of glycine in a gelatin capsule, force fed) Both plasma nonprotein nitrogen and amino nitrogen levels determined at two and four hours after administration of glycine showed that the birds which had received vitamin B_{12} were better able to metabolize the glycine than were the B_{12}-deficient birds. There were evidences of toxicity of glycine in the B_{12}-deficient birds. When food was withdrawn from the chicks the night before the glycine was administered, the glycine was much more toxic to the B_{12}-deficient birds than when they were allowed food, those which had received B_{12} were able to withstand glycine with or without food.

Washington State College
Pullman, Wash.

799 MACHLIN, L. J., DENTON C. A., and BIRD H. R.: *Effect of vitamin B_{12} and folic acid on glycine toxicity in the chick,* Federation Proc. 10 388, March 1951.

"Day-old chicks from dams fed a vitamin B_{12} low ration were placed on diets consisting primarily of corn and soybean meal for 4 to 6 weeks. Glycine was added at levels of 3, 6, and 9% with and without the addition of crystalline vitamin B_{12}. The chicks were able to tolerate up to 6% glycine in the presence of B_{12}, whereas 3% was definitely toxic in its absence. In the second experiment, glycine was added at levels of 6 and 9% and the B_{12} content of the diets was varied. The apparent requirement for B_{12} was increased by the addition of glycine. In the third experiment, a 6% level of glycine was used and B_{12}, folic acid, choline, or a combination of folic acid and choline were added. Folic acid, in the presence of choline, alleviated the glycine toxicity In the 4th experiment, the diets contained B_{12} and folic acid, alone and in combination, with and without the addition of 6% of glycine. Folic acid again helped to counteract the toxicity, although not as much as did the B_{12}. When both were added, the chicks grew almost as well as those receiving no glycine. The uric acid content of the blood was raised either by glycine or B_{12}. It was raised more by the two in combination than by either alone. Folic acid reversed this effect and decreased blood uric acid."

U. S. Department of Agriculture
Beltsville, Md.

800. HARDIN J O., and HOVE, E. L.: *Prevention of DL-methionine toxicity in rats by vitamins E B_{12}, folacin, glycine and arginine,* Proc. Soc. Exper Biol. & Med. 78 728-731, Dec. 1951.

Authors summary "(1) The toxicity of DL-methionine fed to rats at a 2% dietary excess level, was more pronounced in the absence of dietary vit. E, folacin, and vit. B_{12}. The combination of vit. E and folacin was as effective for growth as all 3 vitamins together Without the 3 vitamins, growth in 4 weeks was 32% of the normal, as compared with 54% of normal upon their addition to the diet. Supplements of glycine and arginine in the absence of these vitamins produced growth that was 62% of normal. (2) The methionine toxicity was prevented by molar equivalent supplement levels of glycine plus arginine if the 3 vitamins were added to the diet (or o

normal) but was not prevented in the absence of these factors. (3) Urinary creatine and sensitivity to acute carbon tetrachloride were determined for all rats. (4) complete protection against glycine toxicity was given by dietary methionine with vit. E."

Alabama Polytechnic Institute
Auburn, Ala.

801. SURE, B., and EASTERLING L. *The protective action of vitamin B_{12} against the toxicity of* DL *thyroxine*, J Nutrition 42 221 225, Oct. 1950

Studies in rats revealed that as little as one part of crystalline vitamin B_{12} affords 100 per cent protection against a toxic fatal dose of 666 parts of thyroxine Le. 0.3 mcg. of vitamin B_{12} protects against 200 mcg. of thyroxine.

University of Arkansas
Fayetteville, Ark.

802. ERSHOFF B. H. *Beneficial effects of liver on cortisone acetate toxicity in the rat*, Proc. Soc. Exper Biol. & Med. 78 836-840, Dec. 1951

The toxic effects of large doses of cortisone acetate were largely counteracted in the rat by the administration of desiccated whole liver Liver did not counteract the effect of the drug on the leukocyte count. The beneficial effect of liver was apparently not due to its content of known B vitamins.

Supplements of all known B vitamins (B_{12} at level of 150/Kg. of ration) were without significant effect on the gain in body weight of immature rats fed 100 mg / Kg. of diet of cortisone acetate.

Other levels of cortisone used 200 and 400 mg /Kg. of diet. Females were more severely affected by cortisone than males females benefited some by liver

Emory W. Thurston Laboratories
Los Angeles, Calif

ORGANIC, INORGANIC CHEMICALS

803 POPPER, H., KOCH WESER, D., and SZANTO P B. *Protective effect of vitamin B_{12} upon hepatic injury produced by carbon tetrachloride*, Proc. Soc. Exper Biol. & Med. 71 688-690, Aug. 1949

The administration of vitamin B_{12} (15 mcg. per 100 Gm. of body weight) to rats before acute carbon tetrachloride intoxication inhibits the development of histologic changes, especially fatty metamorphosis and depletion of ribonucleic acid. In addition, the deposition of lipids (determined biochemically) and the bromsulphalein retention is less than in the intoxicated controls. These results may be related to an effect of vitamin B_{12} on cytoplasmatic ribonucleic acid, which has been shown to disappear early in hepatic injury

Hektoen Institute for Medical Research,
Cook County Hospital, and
Northwestern University Medical School
Chicago, Ill.

804 KOCH WESER, D., and POPPER, H.: *Correlation between ribose nucleic acid depletion and other signs of liver damage as influenced by vitamin B_{12}.* Proc. Central Soc. Clin. Research 22 91 92, Nov 1949 J Lab. & Clin. Med. 34 1764-1765, Dec. 1949 (In Soc. Proc.)

Since disappearance of cytoplasmic ribonucleic acid compounds, which supposedly are important to protein formation, is one of the first signs of liver damage, the authors studied ribonucleic acid depletion in 86 rats previously injected intraperitoneally with carbon tetrachloride. The histologic signs of ribonucleic acid depletion were then correlated with other signs of liver damage. Recent investigations had suggested that vitamin B_{12} promotes the formation of ribonucleic acid compounds and it had been shown that the effects of carbon tetrachloride poisoning were modified in rats that had previously received large doses of this vitamin. Therefore, 48 rats thus protected were included in this study Since small doses of carbon tetrachloride produced ribonucleic acid depletion in the center of the lobules without other evidence of liver damage, it appears that ribonucleic acid depletion is one of the basic factors in this type of liver damage. Moreover vitamin B_{12} seems to inhibit liver damage by facilitating the formation of ribonucleic acid. This action is probably related to the supposed role of growth factors such as vitamin B_{12} as synthesizing ribonucleases. Their influence on protein formation may in turn control the enzyme regeneration in liver damage.

In view of these findings, it is felt that the effect of vitamin B_{12} in different types of liver diseases should be investigated. Considering the large doses of this vitamin required to protect rats against liver damage, the variations in B_{12} content of liver extract may explain the erratic results the extract produces in the treatment of liver disorders.

805 KOCH WESER, D., SZANTO P B., FARBER, E., and POPPER, H.: *Further investigation on the effect of vitamin B_{12} concentrate upon hepatic injury produced by carbon tetrachloride*, J Lab. & Clin Med. 36 694-704 Nov 1950.

Bromsulfalein retention, increase in liver weight and increase in total lipids due to CCl_4 was lessened by the administration of 15 mcg. B_{12}/100 Gm. body weight—given in 4 divided doses before administration of CCl_4 small amounts before or large doses after CCl_4 were ineffective.

Histologically there appeared to be less fatty metamorphosis and central necrosis and less loss of pyroninophilia in the rats given vitamin B_{12}.

Prior administration of vitamin B_{12} concentrate increased the lethal dose of CCl_4 significantly action of B_{12} as a pharmacologic vasodilator was considered.

806. MUSHETT C. W *Influence of crystalline vitamin B_{12} on carbon tetrachloride poisoning* Federation Proc. 9 339 March 1950.

"In confirmation of results obtained by Popper and associates (*Proc. Soc. Exper Biol. & Med.* 71: 688, 1949 [Abstr 803]) with vitamin B_{12} concentrate, it has been found that crystalline vitamin B_{12} exerts a protective effect against the fatty metamorphosis, hydropic change and depletion of cytoplasmic ribonucleic acid in the livers of rats given a single oral dose of carbon tetrachloride. However when both carbon tetrachloride and vitamin B_{12} were given daily for a period of 3 weeks vitamin B_{12} failed to prevent the hepatic changes even at a dose level

of 200 μg. In fact, the livers of the B_{12} treated rats were relatively larger and contained more lipid than those of rats given carbon tetrachloride alone. Despite its inability to protect the livers of the poisoned rats, vitamin B_{12} even at low doses, enabled the treated animals to grow at a considerably greater rate than the rats not treated with the vitamin. An explanation for the growth effect was found not to be due to differences in renal damage since this was negligible in both groups. In dogs given repeated doses of carbon tetrachloride vitamin B_{12} failed to show a protective effect during life as judged by morphologic blood picture prothrombin time, icteric index and blood biochemical determinations. The continuation of vitamin B_{12} treatment, however after cessation of carbon tetra chloride dosing apparently brought about a more rapid reversibility of hepatic changes as indicated by gross and microscopic examination of the livers of the dogs."

Merck Institute for Therapeutic Research
Rahway N J

807 HOVE, E. L., and HARDIN J O *Creatine formation in CCl_4 poisoned rats as influenced by dietary α-tocopherol, vitamin B_{12} and cystine* Federation Proc. 10 385, March 1951.

"Rats reared on synthetic type diets with 10% casein were subjected to weekly sub-lethal CCl_4 injections. Supplements of L-cystine, DL-α-tocopherol acetate and vitamin B_{12} in all combinations were added, or the casein was increased to 18% with and without tocopherol. The creatinurea associated with the vitamin E deficiency was doubled by the CCl_4 injections among rats on the 10% casein diet but was not influenced in rats on the 18% casein diet, or in rats on the 10% basal diet supplemented with cystine. Vitamin B_{12} increased the creatinurea except in the presence of both cystine and vitamin E. Determinations were made on the ability of liver slices from rats on the various dietaries to synthesize creatine, *in vitro* from guanidoacetic acid and methionine. Conditions resulting in high creatinurea such as vitamin E deficiency or CCl_4 toxicity were associated with low creatine synthesis by liver slices, except when vitamin B_{12} was included in the diet. In these cases not only was the synthetic activity of the liver increased, but methionine was not necessary in the substrate. The conclusion drawn from the results on growth, growth deficit due to CCl_4, creatinurea, tooth color and other criteria is that the 10% casein diet is made equal to the 18% casein diet by the addition of *both* cystine and vitamin B_{12}. In the absence of these factors the vitamin E requirement of the rat is increased and vitamin E is involved in the synthesis of creatine from guanidoacetic acid and labile methyl."

Alabama Polytechnic Institute
Auburn, Ala.

808. HOVE, E. L., and HARDIN J O: *Effect of vitamins E B_{12} and folacin on CCl_4 toxicity and protein utilization in rats* Proc. Soc. Exper Biol. & Med. 77: 502-505, July 1951.

Authors summary "Vit. E and vit. B_{12} can replace each other in promoting protein utilization and in protecting against acute carbon tetrachloride toxicity in young rats reared on 10% protein diets deficient in these factors. The vit. B_{12} requirement for protection against CCl_4 is considerably higher than for growth. Folacin supplements, alone, had no effect, but, in the presence of vit. B_{12} or vit. E, this factor markedly improved protein utilization and growth. L-Cystine protected against the acute toxicity but a synergism between cystine and vit. E was not noted."

Alabama Polytechnic Institute
Auburn, Ala.

809 HOVE, E. L., and HARDIN J O: *Effect of vitamin E and CCl_4 on fat, respiration and choline oxidase of rat livers*, Proc. Soc. Exper Biol. & Med. 78 858-861 Dec. 1951.

Both vitamin E and vitamin B_{12} have been found to give protection against liver damage induced in rats by carbon tetrachloride. Since carbon tetrachloride is known to produce an immediate fatty infiltration of the liver and an increased rate of respiration of liver slices from guinea pigs poisoned with carbon tetrachloride has been reported by Ennor (*Australian J Exper Biol. & M Sc.* 20 73 1942) the influence of vitamin E and vitamin B_{12} on the response of liver fat and the rate of liver slice respiration after acute carbon tetrachloride poisoning was investigated in rats. The liver fat of rats on a 10 per cent casein diet was significantly lower in animals that received no vitamin E than in those that were given this vitamin, but no difference was noted in the treated and untreated animals when the diet contained 18 per cent casein. Following injections of carbon tetrachloride the liver fat increased sharply to about the same degree on all diets. Although the fatty changes 24 hours after the injections were not affected by the vitamin supplements, these supplements, especially vitamin B_{12}, permitted a more rapid return to normal liver fat values. Increase of respiration of liver slices from animals injected with carbon tetrachlor ide was not substantially inhibited by dietary supplements of vitamin E. The choline oxidase activity of the liver was not influenced by dietary vitamin E or by carbon chloride injections into rats, but increased dietary fat level increased the activity of this enzyme system.

Alabama Polytechnic Institute
Auburn, Ala.

810. ERSHOFF B. H. *Comparative effects of B vitamins and liver on dinitrophenol toxicity in the rat,* J Nutrition 42 271 277 Oct. 1950.

When 0.1 per cent dinitrophenol was added to the diet of rats, retardation in body and testicular weights was produced. These effects were completely counter acted by the administration of desiccated whole liver Supplements of all of the known B vitamins, including vitamin B_{12} had little, if any effect. It is pointed out, however that the vitamin B_{12} content of the whole liver ration was considerably greater than the B_{12} content of the diet containing the synthetic vitamin supplements. It is possible that the protective effects of the whole liver ration may have been partly due to its higher vitamin B_{12} content.

University of Southern California
Los Angeles, Calif.

811. FISHER, B., and VARS, H. M. *Effect of vitamin B_{12} and aureomycin upon the toxicity of chloroform to regenerating liver* Federation Proc. 10 354, March 1951.

"Adult male Wistar rats were fed a non-protein diet for 14 days, subjected to 70% partial hepatectomy and offered an 18% fibrin diet. Group A received 10 mg. aureomycin per gram of food eaten. Group B was injected with 2γ vitamin B_{12} on alternate days (total dose 8γ) and group C received saline injections. On the 4th postoperative day all received a subcutaneous injection of chloroform (1.2 ml/kg.) in mineral oil (1 ml. volume) and were sacrificed 4 days later All groups ate approximately the same amount of food. There was no significant difference between the groups in the amount of liver protein regeneration or in the histological picture as observed by hematoxylin and eosin, or methyl-green and pyronine stains. Varying the time intervals of therapy and sacrifice did not change the results. We conclude that under these conditions neither vitamin B_{12} nor aureomycin exerted either a protective action to the liver or a stimulus to liver protein regeneration."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

812. DiPORTO A., and SEBASTIANO, M. *Action of vitamin B_{12} supplemented by vitamin C and vitamin K in experimental intoxication due to benzene hematological aspects*, Policlinico 58 172 June 1951

813. STOKINGER, H. E., and STROUD M. T: *Anemia in acute experimental beryllium poisoning*, J Lab & Clin. Med. 38 178-182, Aug. 1951.

A mild anemia was produced in dogs, rats, and rabbits by exposing them to an aqueous aerosol of beryllium fluoride. The anemia resembled a macrocytic anemia and differed according to the species in dogs the red blood cell count, mean corpuscular volume, and hemoglobin all changed as in typical normochromic macrocytic anemia in rabbits there was a lessened tendency for the hemoglobin concentration of the whole blood to decrease and the tendency to return to normal was more pronounced in rats the hemoglobin values were normal while the other two values changed as in macrocytic anemia.

Vitamin B_{12} was given to 2 dogs during the period of exposure to the beryllium mist, and to 2 others after 28 weeks of exposures to the mist none of the dogs received any benefit from the vitamin. Another group of dogs was given liver-stomach concentrate for a month before, and during the period of, exposures to the beryllium salt aerosol these dogs fared worse than did the control dogs. Folic acid given at the time the anemia was being induced was successful in treating rats but appeared to contribute to the death of one of the dogs that received it.

In the discussion the authors state "A possible explanation for the detrimental effect of [the liver-stomach concentrate] on the anemia in dogs might be that its iron, a known gastric irritant, further added to the irritant effect of beryllium brought to the gastrointestinal tract by ciliary action in the respiratory system. Whether a similar explanation holds for folic acid is not known."

University of Rochester
Rochester N Y

814 MUSHETT C. W., KELLEY K. L., BOXER, G. E., and RICKARDS J C.: *Antidotal efficacy of vitamin B_{12a} (hydroxo-cobalamin) in experimental cyanide poisoning*, Proc. Soc. Exper Biol. & Med. 81 234-237 Oct. 1952.

Authors summary and conclusions: "1. Vit. B_{12a} (hydroxo-cobalamin) but not vitamin B_{12} (cyano-cobalamin), has been found to be capable of preventing in mice the toxic symptoms and death due to cyanide administration. 2. When injected into mice exhibiting complete respiratory arrest and coma due to cyanide poisoning, vit. B_{12a} effected rapid recovery of most animals. 3. In mice injected with potassium cyanide followed by vit. B_{12a}, some of the cyanide appears in the urine as thiocyanate, but a greater percentage of the cyanide appears as vit. B_{12}, having formed this compound by reacting with the vit. B_{12a}."

Merck Institute for Therapeutic Research, and
Merck & Co., Inc.
Rahway N J

815. ANDREWS, G C., POST C. F., and DOMONKOS A. N. *Seborrheic dermatitis supplemental treatment with Vitamin B_{12}*, New York State. J Med 50 1921 1925, Aug 15, 1950

The authors consider seborrheic dermatitis to be essentially a nutritional disease, due to an underlying digestive deficiency This can be corrected by vitamin B complex and crude liver extract therapy Folic acid has been used, but does not give as good results as crude liver extract. Many patients with seborrheic dermatitis are infected by Pityrosporon or staphylococci. It may be assumed that the resistance of these patients to infection has been lowered by nutritional or metabolic disturbances, which can be overcome by treatment with crude liver extract, vitamin B complex, folic acid, or vitamin B_{12}.

In the past year the authors have used vitamin B_{12} and vitamin B_{12} concentrate parenterally in the treatment of 101 dermatologic patients. The dosage was 10 to 30 mcg. once a week or in some cases, every two to three weeks. Secondary infections of the seborrheic areas were treated with the sulfonamides or penicillin. Local treatment was given as necessary Of the 101 patients, 66 were followed sufficiently for evaluation of the effects of vitamin B_{12}. In 16 of the 37 cases of seborrheic dermatitis, and in each of the single cases of chronic radiodermatitis and psoriasiform dermatitis there was great improvement, usually after two or three injections. Many of these patients have remained entirely free of seborrheic dermatitis. Mild recurrences were usually satisfactorily treated with a few additional vitamin B_{12} injections, and recurrences have been prevented entirely by a maintenance dose of 15 or 30 mcg. every two or three weeks.

Presbyterian Hospital, and
Columbia University College of Physicians and Surgeons
New York, N Y

816. ANDREWS G C., POST C. F., and DOMONKOS, A. N *Advances in the treatment of seborrheic dermatitis and acne vulgaris*, Australian J Dermat. 1 11 March 1951 (abstr Am. Pract. 3 256, March 1952)

Vitamin B_{12} administered intramuscularly in doses of 10 to 30 mcg. once a week or in some cases, once every two to three weeks, as an adjunct to local treatment, has brought about improvement in a large number of patients with seborrheic dermatitis after two or three injections. Many patients have had no recurrences. A mild recurrence can usually be treated satisfactorily with a few additional injections, and recurrences have been prevented entirely by a maintenance dose of 15 or 30 mcg every two or three weeks. The concentrate and the crystalline preparation had similar action. Larger doses are necessary to bring about a response in patients with the skin disease than in those with pernicious anemia.

New York, N Y

817 ANDREWS, G C., DOMONKOS, A. N., and POST C. F *Treatment of acne vulgaris*, J.A.M.A. 146 1107 1113, July 21 1951.

For two years the authors have treated acne vulgaris by means of antibiotics and estrogens and have not employed X ray therapy Results are given for 384 patients, comparison being made with an earlier series of 253 patients who were treated with X rays.

The authors state that patients with acne simplex can usually be cured by a low fat diet, the administration of vitamin A and diethylstilbestrol and local measures. They believe that in pustular nodular and cystic types the patients usually are sensitized to staphylococci and often harbor foci of infection which not only contribute to the pustulation and make it worse, but may be the sole cause. The staphylococci in acne patients differ in their susceptibility to antibiotics. Penicillin usually has little clinical effect and the best results have been obtained with aureomycin, terramycin and sulfadiazine. Some patients who failed to respond to these drugs were cured when dihydrostreptomycin was used.

Treatment usually consisted of several months of oral administration of diethylstilbestrol (in both girls and boys) oral administration of a suitable antibiotic or sulfonamide—terramycin being preferred, and supplementary local treatment. Sometimes the administration of the estrogen could be stopped and progress continued with antibiotics and local therapy Some patients responded well to antibiotics and local therapy only Topical applications of tyrothricin, bacitracin and aureomycin were sometimes beneficial.

In discussing systemic measures advisable in acne patients, it is mentioned that where the patient is under weight, has poor posture, pallor and listlessness, injections of vitamin B_{12} or liver extract are given. These are also useful if there is much seborrheic dermatitis concomitant with the acne. When comedones are present, vitamin A is given, either orally in doses of 100,000 or 200,000 units daily or in severe cases, parenterally in the form of an aqueous solution twice weekly (in addition to the oral vitamin A)

The final results in the 384 patients treated by the antibiotic (or sulfonamide) and estrogen method are as follows: 90 patients (23%) were cured 276 (71%) were improved, and 18 (6%) were unimproved or worse. The observation period has been too short for the number of recurrences to be estimated. In the control series of 253 patients treated with X rays, the results were as follows 152 patients (60%) were cleared up or improved 51 patients (20%) were not appreciably better or worse, and another 50 patients (20%) cleared up for a time, but then their acne returned.

Presbyterian Hospital
New York, N Y

818. AYRES, S., Jr., AYRES, S., III, and MIROVICH, J I *Macrocytic anemia and impaired liver function in eczematous and certain other dermatoses* Arch. Dermat. & Syph. 62 851-872, Dec. 1950

During the past two years the authors have encountered 27 patients with dermatoses of obscure origin in which the manifestations of macrocytic anemia found. Both the skin lesions and the blood picture

gratifying responses to intragluteal injections of crude liver extract containing folic acid, together with high potency vitamin B complex and iron administered orally Vitamin B_{12} was available for treatment of 4 cases Intra gluteal injection of 10 to 15 mcg. once a week has given strikingly good results.

In 88 other patients with various dermatoses, especially of an eczematous type, the liver function was impaired. In the majority of cases cure or improvement followed dietary regulation, administration of lipotropic substances in the form of choline or methischol® (which contains methionine, inositol and choline) of high potency vitamin B complex with vitamin C, of crude liver extract with folic acid given intragluteally in a dose of 2 cc. at five- to seven-day intervals, and symptomatic procedures.

Illustrative case histories are included.

Los Angeles, Calif.

819 DIETERICH, J P *Infantile eczema—therapy with vitamin B_{12} a clinical note* Ann. West. Med. & Surg. 5 47-48, Jan. 1951

Since infantile eczema is generally considered to be of an allergic nature, and since it has been observed that a patient suffering from allergic dermatitis was affected favorably by vitamin B_{12}, the author used it in a 2 year old girl who had had eczema since she was 5 days old. Eliminating cow's milk from the diet had brought about slight improvement before vitamin B_{12} administration was begun. The dosage of vitamin B_{12} was 20 drops (10 mcg.) by mouth daily for one month, then 15 drops daily Within two weeks after this treatment was started, there was a complete remission which has lasted for four months at the time of writing. The child's appetite improved, she gained in weight, and seemed to be developing more rapidly than previously

Glendale, Calif

820. SIMON S. W *Vitamin B_{12} therapy in allergy and chronic dermatoses*, J Allergy 22 183-185, March 1951

Vitamin B_{12} therapy was evaluated in 20 adult patients with asthma. Nineteen had intractable asthma; the remaining patient had mild asthma but severe eczematoid atopic dermatitis. Four intramuscular injections of 1,000 mcg. of vitamin B_{12} were given at weekly intervals. Treatment used prior to B_{12} administration was continued throughout the seven-week period of study It was found that 12 patients gained weight, 6 lost, and 2 remained the same. Vital capacity, which in all but one patient was originally below normal, showed an increase in 14, a decrease in 2, and was unchanged in 4 patients. Hematologic findings were not significantly altered. Subjectively 18 of the patients stated that there was a definite improvement with reference either to asthma, shortness of breath upon exertion, appetite, sleep, or general condition. This improvement was not always associated with an increase in vital capacity was not spectacular and could have been partly due to the institution of new treatment.

The patient who had both asthma and atopic dermatitis had a very definite and spectacular clearing of his chronic dermatitis. This prompted further investigations with B_{12} treatment in 28 patients with chronic dermatoses.

Ten of the patients had atopic dermatitis 1 showed great improvement, 5 showed moderate improvement, and the remaining 4 showed none. Where there was a definite relationship between atopic dermatitis and worry fear or anxiety (neurodermatitis) no result was obtained with B_{12} therapy

Six of the patients had chronic contact dermatitis none of these had improved with local treatment and elimination of the causative agent, but all responded to vitamin B_{12} with great improvement or complete clearing.

Vitamin B_{12} was ineffective in 2 patients with dermatitis herpetiformis.

Of 10 patients with chronic urticaria lasting for one year or more, 9 were greatly improved or cured and one was definitely worse after treatment with vitamin B_{12}. The latter patient was found to have an intestinal infection. When chloramphenicol therapy was instituted, the urticaria cleared.

The authors state that no absolute conclusions can be drawn from the small number of patients treated in each category They believe that vitamin B_{12} is probably of no value in the treatment of chronic bronchial asthma. Further trial of vitamin B_{12} in chronic contact dermatitis and chronic urticaria is, however considered indicated.

Dayton, Ohio

821 LEVY W *Effect of vitamin B_{12} on ichthyosis*, Lancet 2 144, July 19 1952 (in Letters to the Editor)

"Ichthyosis is a distressing condition, so I should like to report a chance observation. A man, aged 69 recovering from a slight cerebral vascular accident, received injections of vitamin B_{12} ('Cytamen 50') 50 µg. twice weekly, as a 'tonic. He has had ichthyosis all his life. After about four injections the scaling appeared to be much reduced, and after ten injections few scales are left. Is this a natural remission?"

London, N.W. 6, England

822. FRIEDMAN R., WEINER, J., and GROPAN J: *Dermatitis herpetiformis* A.M.A. Arch. Dermat. & Syph. 64 381, Sept. 1951 (In Soc. Proc.)

A case of dermatitis herpetiformis was presented for recommendations regarding treatment. The patient had improved dramatically under sulfapyridine treatment but began to develop signs of agranulocytosis. At the time of presentation she was being treated with sulfadiazine.

Various therapeutic regimens were suggested, including vitamin B_{12} which was reported to have been effective in one case, in doses of 1 cc. or 30 mcg.

823 ROSTENBERG A., JR., and PERKINS, A. J: *Nickel and cobalt dermatitis*, J Allergy 22 466-474, Sept. 1951

A patient who was being examined for allergy to metals (later narrowed to nickel, cobalt, and gold) was found to give a delayed tuberculin type reaction to crystal

line vitamin B_{12}. The authors believe that this is the first report of an allergic reaction to crystalline vitamin B_{12}.

University of Illinois College of Medicine and University of Illinois College of Pharmacy Chicago, Ill.

824 ORCHARD W E. *Dermatitis after use of pentavalent arsenicals per vaginam* Brit. M J 2 1444 Dec. 15, 1951

A pregnant woman had an allergic reaction (dermatitis) when treated with suppositories containing pentavalent arsenic for leucorrhea. She was given promethazine hydrochloride, a vitamin B complex, and vitamin B_{12}. The acute skin condition had subsided within a week. The patient had also received penicillin and bismuth compounds for syphilis, but it was determined that neither of these chemicals were at fault in causing the dermatitis. This is a case in which a patient is sensitive to both trivalent and pentavalent arsenic.

825 GOLDBLATT S *Treatment of lupus erythematosus with vitamin B_{12}, preliminary report of 4 cases*, J Invest. Dermat. 17 303-304, Dec. 1951.

Three cases of chronic discoid lupus erythematosus responded well to intramuscular injections of 15 mcg. of vitamin B_{12} weekly. Response was especially good in the case which was of least duration (about six months). A woman with subacute disseminated lupus erythematosus was treated with 15 mcg. of vitamin B_{12} three times a week. Following each injection there was noticeable fading of the lesions, and they began to lose their elevation and induration. Toxic symptoms were eliminated rapidly even though the patient twice exposed herself to excessive sunlight. After one such exposure, the dosage of vitamin B_{12} was increased to 30 mcg. after two injections the sensation of fatigue and dizziness disappeared and the erythema faded rapidly. It is considered probable that larger doses would be more effective. Doses as high as 5,000 mcg. have been reported to cause no toxic symptoms.

826. BLOCK, M. T *Vitamin E in the treatment of diseases of the skin*, Clin. Med. 60 31-34, Jan. 1953.

In 3 patients with lupus erythematosus who responded poorly to vitamin E the lesions were cleared when vitamin B_{12} was used, 1,000 mcg. intramuscularly twice a week for 12 doses. Two cases of leukoplakia, also, were arrested by 1,000 mcg. of vitamin B_{12} given intramuscularly

Newark, N J

827 GAUMOND E.: *Vitaminothérapie à hautes doses en dermatologie (Vitamin therapy in high dosage in dermatology)* Union méd. du Canada 82 141-155, Feb. 1953.

From published reports (about 70 references) and his personal experience the author discusses the therapeutic and pharmacodynamic effect of the various vitamins in dermatology. The results are tabulated

In referring to vitamin B_{12} two cases are cited, one each of lupus erythematosus and seborrheic dermatitis, in which good results were obtained with use of B_{12} 15 mg. one to three times weekly

Quebec, Canada

828. LEE, T H., LERNER, A. B., and HALBERG R. J: *Water soluble vitamins in normal human skin*, J Invest. Dermat. 20 19-26, Jan 1953.

This is a report of a preliminary study on the concentration of vitamin B_{12}, folic acid, riboflavin, niacin, pantothenic acid, biotin, thiamine, and ascorbic acid in the skin of 15 normal humans. Assay procedures are described in detail. The amounts found are shown in tables. In 9 samples, assayed microbiologically an average of 0.021 mcg. of water-soluble vitamin B_{12} per Gm. of dry skin was demonstrated. The authors comment on the role of vitamins in skin as follows

"It has been postulated that skin, like muscle, derives its energy mainly from carbohydrate metabolism. The latter is carried out by glycolysis of carbohydrate and subsequent oxidation of pyruvic acid and acetic acid, which are also reaction products of fat and protein metabolism, to the final end-products, carbon dioxide and water via Kreb's citric acid cycle. Decarboxylation of pyruvic acid to acetic acid requires a co-enzyme which is made up partly of thiamine. Furthermore, nicotinic acid is present in enzymes which catalyze several reactions in carbohydrate metabolism. Riboflavin, pantothenic acid, and biotin also play important roles in the metabolism of either carbohydrate or of protein and fat. The presence of these as well as other vitamins in considerable concentration in human skin is in support of this postulation."

University of Michigan Medical School Ann Arbor, Mich.

Neoplasms

ANIMAL STUDIES

829 OLESON J J., and LITTLE, P A. *Effect of pteroylglutamic acid and vitamin B_{12} on growth of Rous tumor implants*, Proc. Soc. Exper Biol. & Med. 71 226-227 June 1949

Pteroylglutamic acid has previously been found essential for the growth of implants of Rous sarcoma in young chicks. The pteroylglutamic acid can be replaced by 15-unit liver extract, or by highly purified vitamin B_{12} concentrates. The effect of the liver extract is duplicated by as little as 0 05 mcg. a day of vitamin B_{12} higher levels produce larger tumors. When pteroylglutamic acid and vitamin B_{12} are given together the tumors appear earlier and attain greater size than when corresponding levels of either factor are given alone. Thus vitamin B_{12} does not entirely replace pteroylglutamic acid in promoting the growth of the Rous tumor but is required in addition to give maximum tumor growth under these conditions.

830. DAY P L., PAYNE, L. D., and DINNING J S. *Procarcinogenic effect of vitamin B_{12} on p-dimethylaminoazobenzene-fed rats*, Proc. Soc. Exper Biol. & Med. 74 854-855, Aug 1950.

The addition of 5 mcg. of vitamin B_{12} to a methionine-deficient diet containing 70 mg of p-dimethylaminoazobenzene increased the incidence of hepatomas in rats from 17 to 78 per cent. In rats on a similar diet supplemented with methionine the incidence of hepatomas was increased only from 11 to 33 per cent by vitamin B_{12} methionine therefore appears to provide some protection. No tumors occurred in rats on a diet containing B_{12} but no p-dimethylaminoazobenzene. Thus, there is no evidence that B_{12} itself is carcinogenic. Its procarcinogenic effect when given with p-dimethylaminoazobenzene may be a reflection of its influence on metabolic transformation of the carcinogen, or may indicate that this vitamin is required for tumor growth.

University of Arkansas
Little Rock, Ark.

831 ENGEL, R. W., and COPELAND D H. *The influence of dietary casein level on tumor induction with 2-acetylaminofluorene*, Cancer Research 12 905-908, Dec. 1952.

A high (40 to 60%) casein diet gave some protection to rats fed up to 2.1 mg. per rat of the carcinogen 2-acetylaminofluorene. Addition of 30 mcg of vitamin B_{12} and 2 mg. of folacin per Kg. to the diet decreased the average survival period from 42 to 32 weeks in a group of rats fed on the 60% casein diet while receiving 2.4 mg of carcinogen per rat.

Alabama Polytechnic Institute
Auburn, Ala.

832. SHAPIRO D M., and GELLHORN A. *Combinations of chemical compounds in experimental cancer therapy* Cancer Research 11 35-41 Jan. 1951.

Authors summary: "The effect of combinations of chemical compounds in cancer chemotherapy has been investigated. By the use of the guanine analog, 5-amino-7-hydroxy 1H-v-triazolo (d) pyrimidine, together with either desoxypyridoxine, pteroylglutamic acid, 7-methyl pteroylglutamic acid, or vitamin B_{12}, it has been shown that the growth of a transplantable carcinoma of the breast in mice can be inhibited to a greater extent by a combination than by any one of the drugs alone. Evidence has been presented to show that the combinations are not acting by causing host toxicity for the observed effects are associated with minimal to no weight loss. The possible mechanisms of action are discussed.

"It is recognized that in no instance did a combination eradicate the tumor being treated however the possibility of utilizing more effective agents in combination or of increasing the number of active chemical compounds in combination offers an attractive approach to achieve this end."

Columbia University College of Physicians and Surgeons
New York, N Y

833 WOOLLEY, D W: *Evidence for the synthesis of vitamin B_{12} by spontaneous tumors*, Proc. Nat. Acad. Sc. 39 6-18, Jan. 1953.

Mice do not synthesize vitamin B_{12}. If a pregnant mouse ingests a sufficient amount of vitamin B_{12}, enough of this vitamin is passed on to the young to meet almost completely their needs for normal growth, even when the young are fed a B_{12}-deficient diet. This affords a means of determining whether neoplasms synthesize vitamin B_{12} If tumor bearing pregnant mice are kept on a B_{12}-deficient ration but their young grow normally on the deficient diet, the young must have received this vitamin from supplies manufactured by the neoplasm in the mother This was found to be true of the offspring of Swiss mice (and, to a lesser extent, C3H and C strain mice) bearing spontaneous mammary tumors. No evidence was obtained that transplanted mammary tumors of the same strain synthesized vitamin B_{12}. This difference in metabolic behavior between normal and neoplastic tissue is recognized as "a promising pathway to chemotherapeutic attempts." It has been demonstrated previously that specific chemical compounds which are antimetabolites to dimethyldiaminobenzene, a precursor of vitamin B_{12} and riboflavin, harm those living things which synthesize these vitamins but do not affect others. The synthetic ability which the tumors in these experiments possess is akin to the ability of crown galls to synthesize indole acetic acid and that of cambium tissue to form an essential growth factor which neighboring cells require but do not make. In these three instances, undifferentiated tissue differs from differentiated tissue in possessing the ability to synthesize a particular biologically important compound.

Rockefeller Institute for Medical Research
New York, N Y

834 WOOLLEY D W: *Suppression of spontaneous tumors of mice through the considered use of anti metabolites*, Science 117 473, May 1 1953 (In Soc. Proc.)

"The study of the basis of selective action—i.e., of why a given chemical is poisonous to one living thing and harmless to another—has indicated that selective drugs can be predicted and realized through the application of existing biochemical and nutritional knowledge. Thus it can be shown that those cells which synthesize vitamin B_{12} and riboflavin are harmed by suitable anti metabolites, of 1,2-dimethyl-4,5-diaminobenzene, the common precursor of these vitamins. Those cells which do not conduct these syntheses and therefore exhibit a nutritional need for these vitamins, are not affected Because spontaneous mammary tumors of mice have been found to synthesize vitamin B_{12} in contrast to the inability of normal tissues to do likewise, the aforementioned antimetabolites of dimethyldiaminobenzene should selectively harm the neoplasms. Three of these antimetabolites have been shown to cause these spontaneous cancers to become smaller and occasionally to disappear The substances are 1,2-dimethyl-4-amino-5-hydroxybenzene, 1,2-dichloro-4-(p-nitrobenzenesulfonylamido)-5-nitrobenzene, and 1,2 dimethyl-4-(p-carboxyphenylazo-)-5-hydroxybenzene. Not all individuals were benefited, and, in fact, only about 50% of animals exhibited regression or disappearance of the tumors. Furthermore, the regressions of the neoplasms were not permanent. All eventually began to grow again in 1-5 months. Studies were made in an effort to ascertain why the tumors became resistant to the drugs, and why all were not originally susceptible."

Rockefeller Institute for Medical Research
New York, N Y

835 ENGEL, R. W *Influence of diet on spontaneous and nutritionally induced tumors*, Texas Rep. Biol. & Med. 10 974-986, Winter 1952.

Author's summary "The initial observation that the prolonged feeding of diets deficient in choline promotes the production of tumors in the liver and other sites in rats has been amply confirmed by additional studies in our laboratory as well as by workers in several other laboratories. This promotion of spontaneous tumor growth through dietary restriction of choline has also been demonstrated in chickens. No tumors have been observed in dogs in the presently reported studies. The complication of early edema development was also noted to occur in rats when attempts were made to hasten the damaging action of choline-low diets by reducing the dietary protein.

"*It has been pointed out that strain differences in rats may have an important bearing on the results in choline deficiency studies and that at least two strains exhibit markedly different susceptibilities to liver tumor induction by azo dyes. Early results indicate that the striking anticarcinogen action of riboflavin in azo dye carcinogenesis has its parallel in the action of riboflavin in preventing liver tumor induction by the feeding of a low-choline diet.*"

In the experiment with dogs it was found that 6 of the 7 on a choline deficient diet had duodenal ulcers and liver cirrhosis. Two-tenths per cent of dietary choline or a supplement of vitamin B_{12} concentrate protected the livers of control dogs. The author points out that perhaps the high incidence of duodenal ulcers was not directly related to choline deficiency dogs on the deficient diet have variable appetites and inanition may have been a contributing factor

Alabama Polytechnic Institute
Auburn, Ala.

INFECTIONS

836. SAGGESE, V *Oral administration of living cultures of Streptomyces griseus as cure of pertussis and anemia*, Minerva pediat. 1 388, Nov 1949 (abstr A.M.A. Am. J Dis. Child. 83 864, March 1952)

"Excellent clinical results have been obtained by the feeding of living cultures of Streptomyces griseus to patients with whooping cough. Certain types of anemia were also benefited by the same treatment this is explained by the finding that the cultures contain antianemic vitamins."

837 ROBERTS, R. B., and SANDS M.: *The influence of vitamin B_{12} on the growth of bacteriophage T4r* J Bact. 58: 710-712, Nov 1949

This study was made to see if vitamin B_{12} is involved in the multiplication of the virus, bacteriophage T4r Cells of the host, Escherichia coli, strain B, were grown in a synthetic medium. When vitamin B_{12} was added to this medium, the burst time of the virus was decreased and larger bursts were found. It appears, therefore, that vitamin B_{12} is one of the rate-limiting factors in the synthesis of the bacteriophage T4r by Escherichia coli cells in the resting phase.

The authors state that it seems probable that the B_{12} effect on virus multiplication is through its role in thymine synthesis. They give two possible applications of their results: (1) the use of virus growth as a biological assay for vitamin B_{12}, and (2) the use of suitable B_{12}-inactivating or B_{12}-competing agents in virus-infected cells as means of chemotherapy

Carnegie Institution of Washington
Washington, D. C.

838. SULKIN S E., MANIRE, G. P., and ALLEN R. *The influence of vitamin B_{12} on experimental viral infections*, Texas Rep. Biol. & Med. 8 367-368, Fall 1950 (in Soc. Proc.)

The effect of vitamin B_{12} on the course and outcome of experimental infections with the viruses of St. Louis encephalitis and poliomyelitis (Lansing) was studied. No difference was found in mortality rates in the vitamin B_{12}-treated animals and the controls infected with poliomyelitis. Of the animals infected with the St. Louis encephalitis virus, however the B_{12} treated group showed increased susceptibility to this infection. In another experiment with mice infected with the St. Louis encephalitis virus, 82.5 per cent of B_{12}-treated animals were dead by the fifteenth day after inoculation, whereas only 55 per cent of the controls were dead.

Southwestern Medical School of the University of Texas
Dallas, Texas

839 MORGAN, H. R.: *Factors related to the growth of psittacosis virus (strain 6BC) I Pteroylglutamic acid, vitamin B_{12}, and citrovorum factor* J Exper Med. 95 269-276, March 1952.

Author s summary: "The inhibitory action of sodium sulfadiazine on the growth of psittacosis virus (6BC) in embryonated eggs is readily reversed by citrovorum factor but not by small amounts of vitamin B_{12}.

"In embryonated eggs, the pteroylglutamic acid analogues, 9-methylpteroylglutamic acid and 4-aminopteroylaspartic acid, produced some suppression of the growth of psittacosis virus (6BC) 4-Aminopteroylglutamic acid, 4-amino-N^{10}-methylpteroylglutamic acid, and 4-aminopteroylaspartic acid inhibited the growth of this virus in tissue cultures at concentrations which were not toxic for the host tissue. The inhibitory action of 4-amino-N^{10} methylpteroylglutamic acid and 4-aminopteroylaspartic acid was readily overcome by addition of citrovorum factor

"Growth of meningopneumonitis virus in embryonated eggs or tissue culture is suppressed by 4-aminopteroylaspartic acid.

"The advantages of the tissue culture technic for studies on the growth of viruses are discussed."

University of Rochester School of Medicine and Dentistry
Rochester N Y
University of Michigan School of Public Health
Ann Arbor Mich.

840. TAMM, I., FOLKERS K., and HORSFALL, F L., JR. *Inhibition of influenza virus multiplication by 2,5-dimethylbenzimidazole*, Yale J Biol. & Med. 24 559-567 June 1952.

2,5-Dimethylbenzimidazole, the 5,6-isomer of which is a moiety of vitamin B_{12}, is of interest in regard to possible selective inhibitory activity on viral multiplication. Experiments are reported which indicate that this compound inhibits multiplication of influenza viruses through an effect upon the host cell rather than on the virus. Multiplication of influenza B virus (Lee strain) or influenza A virus (PR8 strain) in the chorioallantoic membrane in vitro is inhibited even when dimethylbenzimidazole is given long enough after inoculation so that the process of intracellular multiplication of the agent is well under way The compound does not inactivate the virus nor prevent its adsorption by the membrane, and the membrane retains its capacity to support viral multiplication. Oxygen consumption of the membrane is not affected. Prolonged exposure of the membrane to dimethylbenzimidazole does not diminish the capacity of the membrane to support multiplication of the Lee virus.

Hospital of the Rockefeller Institute for Medical Research
New York, N Y
Merck & C , Inc.
Rahway N J

841. WERTMAN K., and SARANDRIA, J L. *Complement fixing murine typhus antibodies in vitamin deficiency states IV B_{12} deficiency* Proc. Soc. Exper Biol. & Med. 81: 395-397 Nov 1952.

The effect of vitamin B_{12} deficiency on the production of complement fixing antibody for murine typhus rickettsiae was studied in 38 rats in an attempt to explain the increased susceptibility and mortality in malnourished populations exposed to infection.

Authors summary: "This study indicates that a vit. B_{12} deficiency impaired the production of circulating complement-fixing antibody The sera of the inanition control and the ad libitum control animals possessed antibody concentrations of approximately the same titers. This was evident when either 0.0073 mg. N or 0.0365 mg N was employed as the antigen. Therefore, the specific vitamin deficiency and not inanition appears to be the significant factor affecting the production of circulating complement-fixing antibody under these experimental conditions."

University of Pittsburgh
Pittsburgh, Pa.

842. MAY C. D., STEWART C. T., HAMILTON A., and SALMON R. J *Infection as cause of folic acid deficiency and megaloblastic anemia*, A.M.A. Am. J Dis. Child. 84 718-728, Dec. 1952.

Authors summary: "The spontaneous occurrence of megaloblastic anemia in association with infection in infants and in monkeys is described.

"Megaloblastic anemia was induced experimentally in monkeys by producing abscesses with intramuscular injections of turpentine.

"Analyses of the liver for ascorbic acid, vitamin B_{12} and folic acid compounds in natural and experimental infections are presented.

"The low content of folic acid compounds in the liver in both natural and experimental infections and the elimination of megaloblastosis from the marrow by folic acid, but not by vitamin B_{12} or ascorbic acid, leads to the conclusion that infection can cause a deficiency of folic acid compounds.

"The application of these observations to the various types of megaloblastic anemia seen in infants and to treatment is discussed."

Minneapolis, Minn.

Asthma

843. KAUFMAN, R. E. *Effect of vitamin B_{12} in asthma*, Ann. Allergy 9 517-518, July-Aug. 1951

In only one patient, of a small series of 8, was there improvement under vitamin B_{12} therapy

Lenox Hill Hospital
New York, N. Y.

844 POTTENGER, F M., Jr., and KROHN B. *Emergency treatment of the asthmatic with special reference to adrenal cortex and vitamin B_{12}*. Rocky Mountain M. J 49 583-586, July 1952.

The authors discuss emergency treatment of the asthmatic patient, which aims at correcting the disturbances resulting from adrenal exhaustion, and includes use of the adrenal hormones and vitamin B_{12}. There are 2 case histories. The first patient, a 62 year old man, received intravenous adrenal cortical extract, 250 dog units, and 60 mcg. of vitamin B_{12} for immediate relief of distress. His regimen included oral adrenal cortical extract from 10 Gm. of gland, and salt, three times daily A recurrence of asthma over a month later was treated with 300 mg. of cortisone daily gradually reduced to 50 mg. over two weeks. For two succeeding attacks he received corticotropin, 80 mg daily initially gradually reduced to 20 mg daily Corticotropin was given rather than cortisone because the patient's blood pressure had risen. The authors felt that cortisone and corticotropin did not produce any better results than the smaller doses of crude adrenal extract. The second patient, a 67 year old woman, received intramuscularly 250 dog units of adrenal cortical extract daily for 10 days, and responded well.

Another attack a year later was treated successfully with adrenal cortical extract, 500 dog units, and 60 mcg of vitamin B_{12}. In general, adrenal cortical extract in a dose of 50 to 250 dog units is helpful in stopping the attack, and may also combat coincident respiratory infection. The most severe cases are helped by an intravenous drip containing adrenalin, 500 to 1,000 dog units of crude adrenal extract (depending on degree of exhaustion of the patient) 60 mcg. of vitamin B_{12}, and calcium gluconate. Besides its hematopoietic action, which aids oxygenation, B_{12} appears to extend the action of the adrenal cortical hormones, and also promotes lipid metabolism, thus contributing to the general welfare of the patient.

Monrovia, Calif

845 CARUSELLI, M. *Therapy of asthma with vitamin B_{12}* Riforma med. 66 849- Aug. 2, 1952 (abstr J.A.M.A. 150 1731, Dec. 27 1952)

"Good results were obtained with vitamin B_{12} in 10 of 12 patients with asthma to whom the vitamin was given daily in doses of 30 mg. for 15 to 20 days. In six of the patients, the disease was due to bacterial allergy Symptoms improved, the asthmatic attacks ceased, and there was a pronounced improvement in general condition, with weight gains. This therapy also cured severe constipation in one patient and dyspepsia due to hyperacidity in another An antiallergic action of the vitamin is suggested. A recurrence in two patients after three and eight months, respectively subsided when the vitamin was given again. Two patients in whom asthma was associated with circulatory decompensation and whose condition was aggravated by strophanthin and digitalis improved with vitamin B_{12}. This result may be due to an improvement in hemodynamics and oxygenation of the blood or more likely to a direct action of the vitamin on the nutrition and function of the myocardium. The two patients in whom this therapy failed were old persons in whom asthma was associated with severe pulmonary emphysema and hypertensive arteriosclerosis. In these patients the disease may no longer have been due to active allergic factors but to irreversible anatomic and functional changes."

Blood and Cardiovascular

846. BARNARD R. D.: *Indiscriminate transfusion a critique of case reports illustrating hypersensitivity reactions*, New York State J Med. 51 2399-2402, Oct. 15, 1951.

The author reports 4 cases in which the patients were hypersensitive to blood transfusions. Three of the patients died as a result of transfusion. In one of these patien who was leukemic, animal protein factor and terra

Author's summary: "1) These data show that B_{12} increased the tensile strength of wounds during the early phases of healing in rats fed balanced diets containing 19% or 25% protein. This effect was evident at least by the third day in the wounds studied, and it was most noticeable by the sixth day 2) The wounds that were studied in the saline control and vitamin treated rats from the third to eighth day showed no significant difference in their healing rates at the eighth day 3) The delayed healing rate and increased incidence of wound infection in rats that were protein depleted by diet were not significantly altered by administration of vit. B_{12}."

Columbia University College of Physicians and Surgeons, and Presbyterian Hospital
New York, N Y

856. FINDLAY C. W., JR.: *Vitamin B_{12} accelerates wound healing in rats*, Mod. Med. 21 131, July 1, 1953 (in Late Reports from Medical Centers)

"Vitamin B_{12} accelerates wound healing in rats, especially in early stages, probably by taking part in protein synthesis. With a fair to good supply of dietary protein, tensile strength of wounds was increased at least by the third postoperative day and notably by the sixth. Dr Charles W Findlay Jr., produced as good results with treatment after surgery as when preoperative doses were also given. Healing did not improve in animals on a low protein diet."

Columbia University
New York, N Y

General

857 ELVEHJEM, C. A.: *The vitamin B complex*, J.A.M.A. 138 960-971 Nov 27, 1948.

A revision of chapter 11 in the first "Handbook of Nutrition" presents excellent reviews of the factors of the vitamin B complex, including B_{12} (p. 971)

858. ANNOTATION *Further studies on vitamin B_{12}*, Nutrition Rev 6 291 293, Oct. 1948.

859 DAMESHEK, W: *And now B_{12}!* Blood 4: 76-78, Jan. 1949 (Editorial)

Commenting on the extraordinary potency of vitamin B_{12}, the author expresses himself as follows: "In these crowded days when one therapeutic miracle succeeds another in rapid succession, the appearance of a new substance with almost incredible therapeutic effects inspires but little excitement. The isolation of vitamin B_{12} in the research laboratories of Merck and Company in this country and almost simultaneously in the Glaxo Laboratories in England is the most recent case in point. Here is a substance that, when given to a patient suffering from pernicious anemia, results in a maximal reticulocyte response following a single injection of 5 to 10 thousandth of a milligram (0.000005 Gm.)! Has there ever been in the history of medicine a more potent material, microgram for microgram?"

Comparison is made between vitamin B_{12}, folic acid, and liver extract in the treatment of the various anemias, but at the time the article was written any conclusions were necessarily tentative. Evidence from the literature is presented which suggests the identity of vitamin B_{12} with the "animal protein factor" present in crude materials such as fish meal, cow manure, and liver

860. AMYOT R. *La vitamine B_{12} serait-elle la principale substance antianémique des extraits de foie? (Is vitamin B_{12} the main antianemic substance in liver extract?)* Union méd. du Canada 78 64-65, Jan. 1949

The author reviews the work done on vitamin B_{12} and concludes that this vitamin might be the only active substance in liver extract.

861. WOODS, R.: *The story of vitamin B_{12}* Borden's Rev of Nutr Res. 10 1 12, Jan. 1949

In this review article, progress in treatment of pernicious anemia is traced, beginning in the 1920's when it was first recognized that the disease was a manifestation of vitamin deficiency. Contributions of the numerous clinicians and other investigators leading to the isolation and identification of vitamin B_{12} are discussed.

862. LEADING ARTICLE *Vitamin B_{12}*, Lancet 1 151 152, Jan. 22, 1949

This editorial reviews the literature on vitamin B_{12}, and concludes with the following sentence "Meanwhile [until the chemical constitution of vitamin B_{12} is known and its properties established] the practitioner will be well advised to keep to the recognized methods of treatment—with liver extracts that have been clinically tested before issue, or if treatment by mouth is preferred, desiccated stomach."

863 EDITORIAL *Vitamin B_{12}*, Minnesota Med. 3[illegible] June 1949

864 ANNOTATION: *Oral B_{12} seen as possibility* Trade News 24 35, Aug. 8, 1949

Investigations conducted by workers of the [University] of Michigan Hospital indicate that an extract [of] intestine may cause utilization of orally administered vitamin B_{12}. This effect is thought to be identical with the B_{12} potentiating effect of normal human gastric [juice]. Vitamin B_{12} and Castle's "extrinsic" factor are [identical] or closely related.

865 LEADING ARTICLE *Progress with vitamin B_{12}*, [Lan]cet 2 565-566, Sept. 24, 1949

Review article.

866. TASTALDI H. *Factor antianêmico do f[ígado e] vitamina B_{12} (The liver factor and vitamin [B_{12}])* Arq Biol. (Sao Paulo) 33 131 142, 1949

A comprehensive review on the liver factor and vitamin B_{12} with 149 references.

867 CORDIER, R. *Contribution a l'étude de la [vita]mine B_{12} (à propos de 9 observations person[nelles]) (Contribution to the study of vitamin B_{12} [9] personal observations])* Thesis, Imprimer[ie] Beaux-Arts, Lyon, 1949 (review Presse m[éd.] 76, Jan. 25, 1950)

Accounts of the first treatments with vitamin [B_{12} in] France are given (in the original, not in the review). [Indi]cations, criteria of action, and dosage of vitam[in B_{12}] are discussed. The importance of preventing recu[rrence] by injections of 10 mcg. of vitamin B_{12} at interv[als de]termined by the individual case is stressed. The [associa]tive use of gastric juice or acidification of the c[ontents] of the stomach to increase the action of vitamin [B_{12} is] mentioned. Sources of vitamin B_{12} other than liv[er are] discussed.

868. GIRDWOOD R. H. *Vitamin B_{12} and relat[ed fac]tors a clinical and experimental review* Edin[burgh] M. J 57 72 109 Feb. 1950 (abstr Blood [...] Jan. 1951)

"This useful review mentions work on the liv[er fac]tor extrinsic and intrinsic factor yeast and yeast [prod]ucts and folic acid. Animal factors related to vitam[in B_{12}] are discussed; also methods of assay and procedure[s used] in isolation of B_{12}.

"Animal experiments designed to produce a m[egalo]blastic anemia are reviewed with their response to p[teroyl]glutamic acid and B_{12}. The clinical application of vi[tamin] B_{12} is described.

Part II

Analytical Studies of Vitamin B_{12}

Analytical Studies

VITAMIN B₁₂: IDENTIFICATION ISOLATION

891. SHORB M. S.: *Unidentified essential growth factors for* Lactobacillus lactis *found in refined liver extracts and in certain natural material,* J Bact. 53 669 May 1947 (in Soc. Proc.)

Lactobacillus lactis failed to grow in an amino acid basal medium containing all of the synthetic B vitamins or when the medium was supplemented with either clarified tomato juice or certain preparations of liver extracts, but grew when the tomato juice and liver preparations were added together An assay method was developed for each factor The liver factor was found in high concentrations in refined liver extracts and in lower amounts in Wilson liver fraction L, brewers yeast, liquid skim milk, pepsin, erepsin, unclarified tomato juice, bacto yeast extract, autolyzed yeast, trypsin, peptone, tryptone, tryptose, and peptonized milk. These materials had some tomato juice factor activity Crude casein, calcium caseinate, alcohol-extracted casein, acid-hydrolyzed "vitamin free" casein, tryptic digests of crude or "vitamin-free casein, zinc insulin crystals, and chymotrypsin were inactive for the liver factor but had tomato-juice factor activity In sulin, chymotrypsin, and some tomato juices also showed some inhibitory action. Phosphopeptone, ascorbic acid, yeast, nucleic acid, sodium salt of thymonucleic acid, and adenylic acid were inactive for both factors. A third factor was synthesized. The liver factor does not appear to be identical with any of the unidentified growth factors for bacteria in distribution and properties. The other factor(s) may be related to certain bacterial and animal growth factors.

U. S. Department of Agriculture
Washington, D. C.

892. SHORB M. S.: *Unidentified growth factors for* Lactobacillus lactis *in refined liver extracts* J Biol. Chem. 169 455-456, July 1947

It has been found that Lactobacillus lactis Dorner requires two unidentified factors for growth in an amino acid basal medium containing all the synthetic B vitamins (*J Bact.* 53 669 May 1947 [Abstr 891]) One factor (TJ) is *present in clarified canned tomato juice and, in low* amounts, in casein, as well as in many other substances the second (LLD) is found in high concentrations in liver extracts which are active for rat growth, but not in casein or casein hydrolysates. Assays for the LLD factor in crude and refined liver extracts of the type used for intramuscular injection in the treatment of pernicious anemia show that the LLD factor is concentrated in the refined extracts in almost linear relationship to the potency of these extracts in pernicious anemia. This suggests that the LLD factor may be the principle which acts against this disease. The TJ factor is usually more concentrated in crude than in refined liver extracts, and may or may not be active in the treatment of anemias. A third factor appears to be synthesized by L. lactis Dorner Inhibitory substances are present in many natural materials L. lactis Dorner should be of value in the study of these factors and their relation to anemias.

893. RICKES, E. L., BRINK, N. G., KONIUSZY F R., WOOD T R., and FOLKERS K. *Crystalline vitamin B_{12}*, Science 107 396-397 April 16, 1948.

A crystalline compound, tentatively designated as vitamin B_{12}, which in microgram quantities produces a positive hematologic response in addisonian pernicious anemia, has been isolated from liver Dr Mary S Shorb, at the University of Maryland, found that the microorganism Lactobacillus lactis Dorner required two unidentified growth factors one of which appeared to be related to the activity of commercial liver preparations used in the treatment of pernicious anemia. (Abstr 891 892.) Further work on the purification of liver concentrates, which had been in progress at the Merck Research Laboratories for some time, led to the isolation of a crystalline compound which was highly active for the growth of L. lactis (LLD factor) Compared with an arbitrarily selected standard liver concentrate assigned a potency of 1,000 LLD units per mg., the new compound, which crystallizes in the form of minute red needles, has a potency of about 11,000,000 LLD units per mg.

Preliminary clinical tests indicate that vitamin B_{12} has extremely high biologic activity A single intramuscular dose of 150 micrograms produced a very strong hemopoietic response in 1 patient with pernicious anemia, and doses of 3 and 6 micrograms, respectively induced a prompt increase in circulating reticulocytes red cells and hemoglobin in 2 other pernicious anemia patients. If this compound is the only therapeutically active substance *present in commercial liver extracts,* clinical and hematologic responses should be obtained from the parenteral administration of about 1 microgram of vitamin B_{12} daily Approximate vitamin B_{12} contents of several commercial liver extracts for parenteral use are compared.

	Vitamin B_{12} Content (Microbiologic Assay)			
Liver extract	LLD units/cc.	Microg./cc.	Microg./U.S.P. unit	% dry weight
Company A	72,000	4.5	0.4	[illegible]
Company B				
Sample 1	13,000	1.3	0.1	[illegible]
Sample 2	20,000	1.7	0.1	
Company C				
Sample 1	134,000	14.0	0.6	0.014
Sample 2	60,000	6.0	0.5	[illegible]
Company D				
Sample 1	27,000	2.6	0.3	[illegible]
Sample 2	20,000	1.4	0.3	[illegible]

894 SMITH, E. L.: *Purification of anti-pernicious anemia factors from liver* Nature 161 638-639 April 24, 1948 (Letter to Editor)

Two red pigments, both highly active in pernicious anemia, have been isolated from ox liver and from a commerical proteolyzed liver extract. More than 80 clinical tests were made during the fractionation procedures. Activity and color proved to be inseparable. The minimum effective single dose of the nonproteolyzed liver substance has been estimated at about 0.6 mg., while

teolyzed liver yielded a fraction effective at 0.3 mg., although this was not fully purified (since only 25 per cent of the original clinical activity was recovered) The latter fraction was effective in 3 cases of subacute combined degeneration of the spinal cord. Ten batches of red materials all proved clinically active in pernicious anemia (26 cases in all) Further purification of the proteolyzed liver fraction has yielded a product which has eight times its intensity of color This substance has not yet been tested clinically, but if activity remains proportional to color it should be effective at 0.04 mg. The characteristics of the substances are described. It is concluded that they are differing forms of the classical liver factor originally postulated by Minot and Murphy (*Addendum* After this letter was written, the writer was informed of the isolation of crystalline vitamin B_{12} by Merck & Company investigators.)

895. SMITH, E. L., and PARKER, L. F J *Purification of anti-pernicious anaemia factor* Biochem. J 43 viii, May 29 1948 (in Soc. Proc.)

896. ANNOTATION: *Anti-anaemic substances from liver* Lancet 1 876, June 5, 1948.

Mr E. Lester Smith, of Glaxo Laboratories, has crystallized the anti-pernicious anemia factor which he has isolated from liver In a report to the Biochemical Society he said that the crystals obtained resemble those of vitamin B_{12} as illustrated by American workers, and that calculations from color intensity suggest a minimum effective dose of the same order

897 SMITH, E. L., and PARKER, L. F J: *Vitamin B_{12} isolated in England*, Chem. & Eng. News 26 2218, July 26, 1948.

Shortly after the announcement in the United States of the discovery of an anti-pernicious anemia factor called vitamin B_{12} by Merck & Co., Inc., a similar announcement was made by Glaxo Laboratories, Ltd., of England. The English workers reported at a meeting of the Biochemical Society at Oxford that they had concentrated about 1 Gm. of the product from 4 tons of ox liver by means of partition chromatography Red crystals very similar to those exhibited by the Merck group were obtained.

898. RICKES, E. L., BRINK, N G., KONIUSZY F R., WOOD T R., and FOLKERS K. *Comparative data on vitamin B_{12} from liver and from a new source*, Streptomyces griseus, Science 108: 634-635, Dec. 3, 1948.

Milk powder beef extract, and culture broths of strains of Mycobacterium smegmatis, Lactobacillus arabinosus, Bacillus subtilis, and of several Streptomyces species (S. roseochromogenus, S. griseus, and S. antibioticus) were found to promote the growth of Lactobacillus lactis. The properties of a red crystalline compound isolated from one of these sources, a grisein-producing strain of S. griseus, were found on test to be similar to those reported for vitamin B_{12}. Like vitamin B_{12}, the crystals contain cobalt and phosphorus. They have comparable activity for the growth of Lactobacillus lactis, and show "animal protein factor" activity for the chick equal to that of vitamin B_{12}. Randolph West has tested the crystals clinically (personal communication) and has found that the response in pernicious anemia parallels that obtained with vitamin B_{12}. These findings are evidence that the crystals from the microbiologic source and vitamin B_{12} are identical.

899 ELLIS, B., PETROW V., and SNOOK, G. F *The isolation of the crystalline antipernicious anemia factor from liver* J Pharm. and Pharmacol. 1 60-61 Jan. 1949

900. SMITH, E. L. *Crystalline anti-pernicious-anaemia factor* Brit. M. J 2 1367 1369 Dec. 17 1949

This is a review of work that led to the isolation of crystalline vitamin B_{12} at Glaxo Laboratories, with comment upon the value of the vitamin for standardization of liver preparations, the chemical nature of B_{12}, and production of a purified concentrate from Streptomyces griseus fermentation liquors by the Merck group.

Glaxo Laboratories, Ltd.
Greenford, Middlesex, England

901 FANTES K. H., PAGE, J E., PARKER, L. F J., and SMITH, E. L. *Crystalline anti-pernicious anaemia factor from liver* Proc. Roy Soc. 136 592-613, Jan. 1950.

A red crystalline substance has been isolated in a very small yield from ox liver; it has been shown to have intense activity against pernicious anemia. It is probably identical with vitamin B_{12} isolated a few weeks previously in America. The procedures by which it was isolated are reported, as are the results of a number of physical measurements and chemical analyses of the substance.

A second factor which is also active clinically and microbiologically has been separated, but has not yet been crystallized.

From clinical experiments it appears that the minimum effective single dose in pernicious anemia is 10 mcg. or even only 8 mcg. of the crystalline factor with a daily requirement of about 0.5 mcg. It is evident that the anti-pernicious anemia factor is more potent than any known vitamin or hormone. This substance is able to check and, to some extent, reverse the neurologic sequelae of pernicious anemia to the same extent as crude liver extract. It is suggested that the crystalline anti-pernicious anemia factor together with the other red factor may be the only effective antianemic substances present in refined liver extract.

Glaxo Laboratories, Ltd.
Greenford, Middlesex, England

902. HODGKIN, D., PORTER, M. W., and SPILLER, R. C. *Crystallographic measurements on the anti pernicious anaemia factor* Proc. Roy Soc. 136 609-613, Jan. 1950.

Five different samples of the crystalline anti-pernicious anemia factor were examined by various crystallographic technics. They were shown to contain the same molecular structure. The morphology of the crystals is described and certain optical measurements are given. From X ray data it was determined that the molecular weight of the anti-pernicious anemia factor is between 1360 and 1575.

University of Oxford
Oxford, England

CHARACTERIZATION

903. SMITH E. L.: *Presence of cobalt in the anti-pernicious anaemia factor* Nature 162 144-145 July 24, 1948 (Letter to Editor)

The presence of cobalt has been detected in the anti-pernicious anemia factor recently isolated by Glaxo Laboratories by colorimetric estimation, crystals dried in vacuo at 56 C. contained 4 per cent. If each molecule contains one atom of cobalt, the molecular weight of the compound, allowing for 8 per cent loss on drying, is 1,600. The molecular weight of air-dried material by X-ray crystallography is 1,550 to 1 750 (the amount of available material was inadequate for accurate determination of density) The value by the diffusion method is about 3,000. Cobalt has been shown to be effective in a condition involving anemia in ruminants feeding on pastures deficient in this essential trace element. Analytical figures indicate that the molecule also contains three atoms of phosphorus.

904 RICKES, E. L., BRINK, N G., KONIUSZY F R. WOOD T R., and FOLKERS K.: *Vitamin B_{12}, a cobalt complex*, Science 108 134, Aug. 6, 1948.

Spectrographic analysis of Vitamin B_{12} has revealed the presence of cobalt. The vitamin appears to be a cobalt coordination complex which, having six groups about the cobalt atom, could involve one or more organic moieties. The red color of B_{12} is apparently associated, at least in part, with its cobalt-complex character Cobaltous ion (1 mcg./cc.) had no activity for Lactobacillus lactis, for which B_{12} is a highly potent growth factor 0.000013 mcg./cc. promoting half maximum growth. It was also ineffective in 2 cases of pernicious anemia although it was given in excess of the estimated average adult daily dietary intake of cobalt. Phosphorus is also present in B_{12}, as well as nitrogen, although tests for sulfur have been negative.

Microbiologic assay has shown that B_{12} in aqueous solution withstands autoclaving for fifteen minutes at 121 C., that in sodium hydroxide solution at room temperature it is inactivated 20 to 95 per cent in 40 minutes to 95 hours, and that in hydrochloric acid solution at room temperature it is inactivated 18 to 89 per cent in 3 to 95 hours.

905. BRINK, N. G., WOLF D E., KACZKA, E., RICKES E. L., KONIUSZY F R., WOOD T R., and FOLKERS K. *Vitamin B_{12}. IV Further characterization of vitamin B_{12}*. J Am. Chem. Soc. 71 1854-1856, May 1949

Further observations on the physical and chemical characterization of vitamin B_{12} are described. The molecule of vitamin B_{12} possesses one cobalt atom and one phosphorus atom. Vitamin B_{12} is levorotatory The molecule is not a peptide, since hydrolysis of the vitamin does not liberate α-amino acids. Alkali fusion of vitamin B_{12} forms products which react with p-dimethylaminobenzaldehyde characteristic of certain cyclic five-membered nitrogen-containing compounds including pyrroles.

906. ELLIS B., PETROW V., and SNOOK, G F: *The chemistry of antipernicious anaemia factors. Part I The liberation of phosphorus as phosphate from vitamin B_{12}* J Pharm. and Pharmacol. 1 287 291 May 1949

It has been found that acid hydrolyzates of vitamin B_{12} contain phosphate. The atomic ratio of Co P in B_{12} is 1 1 From a comparison of this proportion with the values recorded for the anti-pernicious anemia factor of Smith and Parker it is concluded that this factor is not identical with vitamin B_{12}.

907 SMITH, E. L. *Vitamin B_{12}*, J Pharm. and Pharmacol. 1 500, July 1949

The crystalline anti-pernicious anemia factor isolated by Glaxo workers, and vitamin B_{12}, isolated by Merck workers, are identical in the following respects cobalt and phosphorus content, microbiological activity ultra violet and visible absorption spectra; single crystal x-ray diagrams refractive indices polarographic step behavior on paper chromatograms; clinical potency

908. CUTHBERTSON W F J: *Biochemistry 2 Haemopoietic factors (Folic acid and vitamin B_{12})* Ann. Rep. Chem. Soc. 46 229-244, 1949

Review article 184 references.

909 SMITH, E. L. *Folic acid vitamin B_{12} and anaemia. I Chemical aspects* J Pharm. and Pharmacol. 2 409-417 1950.

Review article.

910. CHAIET L., ROSENBLUM, C., and WOODBURY D T: *Biosynthesis of radioactive vitamin B_{12} containing cobalt*[60] Science 111 601-602, June 2, 1950.

The authors report the preparation of highly pure, crystalline, radioactive vitamin B_{12} by the addition of radioactive cobalt[60] sulfate to a fermentation broth inoculated with Streptomyces griseus.

Merck & Co., Inc. Rahway N J

911 SMITH, E. L.: *Vitamin B_{12}*, Lancet 2 407 Sept. 23, 1950 (in Soc. Proc.)

At the International Congress of Hematology it was reported that the molecular weight of vitamin B_{12} is now estimated at 1650 ± 100 various parts of the molecule have been identified but much of it, including the site of cobalt attachment, remains unknown. Three other clinically active related factors have been identified, but their chemical differences have not been established.

912. BRINK, N G., KUEHL, F A., JR., and FOLKERS K. *Vitamin B_{12} the identification of vitamin B_{12} as a cyano-cobalt coordination complex*, Science 112 354, Sept. 29 1950.

The authors have found that vitamin B_{12} contains one cyano group bound coordinatively to the cobalt

Vitamin B_{12a} does not contain this group. Vitamins B_{12a} and B_{12b} are apparently identical. Since vitamin B_{12} is not toxic, the cyano group must be tightly bound within the coordination complex. Doses as large as 1600 mg./Kg. of body weight, given both intraperitoneally and intravenously to mice, produced no deaths or toxic symptoms. This dose level corresponds to 112,000,000 times the daily human dose of 1 mcg. of vitamin B_{12} listed in the U S Pharmacopeia.

Merck & Co., Inc.
Rahway N J

913 BEAVEN G H., HOLIDAY E. R., JOHNSON E. A., ELLIS B., and PETROW V *A mode of linkage of component α in vitamin B_{12}*, J Pharm. and Pharmacol. 2 733, Oct. 1950 (in Letters to the Editor)

In addition to absorption spectra studies, reference is made to the action of cyanide on the spectrum of B_{12} and the conclusion that "the reversible formation of a purple 'B_{12}-cyanide complex is dependent upon co-ordination of cyanide ion with cobalt."

London, England

914 BEILER, J M., MOSS J N., and MARTIN G J: *Formation of a competitive antagonist of vitamin B_{12} by oxidation*, Science 114 122-123, Aug. 3 1951.

Treatment of vitamin B_{12} in a strong acid solution with hydrogen peroxide was found to form a reaction product that was competitively antagonistic to B_{12}. To 10 cc. of B_{12} solution was added 5 cc. of concentrated HCl. A few drops of a 30 percent solution of hydrogen peroxide was then added and the solution was stirred at room temperature. The solution decolorized, and after standing an hour was neutralized with sodium hydroxide. When the activity was assayed on microorganisms, it was shown to have an inhibitory effect that could be counteracted by B_{12}. When tested on organisms that do not need preformed vitamin B_{12}, no inhibitory effects were observed. The chemical structure of the antagonist is not known, but the cyanide-cobalt complex of B_{12} was believed attacked.

The National Drug Co.
Philadelphia, Pa.

915. DAVIS, B. D *Aromatic biosynthesis. III Role of p-aminobenzoic acid in the formation of vitamin B_{12}*, J Bact. 62 221 230, Aug. 1951.

916. ALICINO J F *Perchloric acid salt of vitamin B_{12}*, J Am. Chem. Soc. 73 4051, Aug. 1951.

Its composition seems to be $C_{63}H_{84}N_{14}O_{14}PCo \cdot 6HClO_4$.

Degradation, Synthesis

917 BRINK, N G., and FOLKERS, K.: *Vitamin B_{12}. VI 5,6-Dimethylbenzimidazole a degradation product of vitamin B_{12}*, J Am. Chem. Soc. 71 2951, Aug. 1949 (Letter to Editor)

Degradation of vitamin B_{12} by acid hydrolysis has given a new basic compound which has been identified by its reactions and by synthesis as 5,6-dimethylbenzimidazole.

918. ELLIS, B., PETROW V., and SNOOK, G F *The "ninhydrin-reacting" hydrolytic fragment of vitamin B_{12}*, J Pharm. and Pharmacol. 1 735. Oct. 1949

The present study indicates that the "ninhydrin-reacting" substance reported earlier is probably 2-aminopropanol.

919 HOLIDAY E. R., and PETROW V: *Vitamin B_{12} as a 5·6-dimethylbenziminazole derivative*, J Pharm. and Pharmacol. 1 734-735, Oct. 1949

Hydrolytic fragments of vitamin B_{12} have been identified with 5,6-dimethylbenziminazoles.

Medical Research Spectrographic Unit, and
British Drug Houses
England

920. BEAVEN G H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., MAMALIS, P., PETROW V., and STURGEON B. *The chemistry of anti-pernicious anaemia factors. Part III 5·6-disubstituted benziminazoles as products of acid hydrolysis of vitamin B_{12}*, J Pharm and Pharmacol. 1 957 970 Dec. 1949

The presence of three chemically related substances (components α, β and γ) in vitamin B_{12} acid hydrolysates has been established. Components α and β are 1, 5,6-trisubstituted benziminazoles (1-substituted 5,6-dimethylbenziminazoles) and γ a 5,6-disubstituted (5,6-dimethyl) benziminazole. On acid hydrolysis only one 5,6-dimethyl benziminazole residue is released from B_{12}. The benziminazole nucleus exists preformed in the vitamin and α, β, γ represent successive stages of degradation of a common precursor The biogenesis and structure of B_{12} is discussed.

921 ELLIS, B., PETROW V., and SNOOK, G F *The chemistry of anti-pernicious anaemia factors. Part II The "ninhydrin-reacting" hydrolytic fragment of vitamin B_{12}*, J Pharm. and Pharmacol. 1: 950-956, Dec. 1949

Earlier findings that hydrolysis of vitamin B_{12} yields a "ninhydrin-reacting" substance have been confirmed. This substance is identical with 2-aminopropanol. Evidence has been obtained for the presence in acid hydrolyzates of vitamin B_{12} of material showing selective absorption with bands and inflections at 2850 2768, 2690, 2585 and 2500 A (referred to as "the 285 component") This "285 component" is resolved into at least two structurally related substances by chromatography employing n-butyl alcohol-acetic acid as the irrigation solvent. Formation of ammonia occurs during acid or alkaline hydrolysis of vitamin B_{12}.

922. BRINK, N G., HOLLY F W., SHUNK, C. H., PEEL, E. W., CAHILL, J J., and FOLKERS, K. *Vitamin B_{12}. IX 1-α D-Ribofuranosido-5,6-dimethylbenzimidazole a degradation product of vitamin B_{12}*, J Am. Chem. Soc. 72 1866, April 1950 (Communication to the Editor)

1-α D-Ribofuranosido-5,6-dimethylbenzimidazole has been obtained by degradation of vitamin B_{12} and by

synthesis. For convenience the names α and β ribazole have been designated for the corresponding 1 D ribofuranoside-5,6-dimethylbenzimadazoles.

Merck & Co., Inc.
Rahway, N. J.

923. WOLF D E., JONES W H., VALIANT J., and FOLKERS, K.: *Vitamin B_{12}. XI Degradation of vitamin B_{12} to Dg 1-amino-2-propanol*, J Am. Chem. Soc. 72 2820, June 1950 (Communication to the Editor)

Degradation of vitamin B_{12} by acid hydrolysis has yielded Dg 1-amino-2 propanol which was characterized by structure examination and by synthesis.

Merck & Co., Inc.
Rahway, N. J.

924 BUCHANAN J G., JOHNSON A. W., MILLS J A., and TODD A. R.: *The isolation of a phosphorus-containing degradation product from vitamins B_{12} and B_{12c}*, Chem. & Indust., p. 426, June 3, 1950.

Studies of the acid hydrolysis (a h) of vitamin B_{12} and vitamin B_{12c} are reported B_{12c} is the "unnamed factor" (Vitamin B_x mentioned by E. Lester Smith *et al.*) Its approximate formula is $C_{63}H_{87}O_{22}N_{14}PCo$ and it is biologically and clinically similar to B_{12}. The difference between B_{12} and B_{12c} lies in the cobalt-containing colored fraction, since the colorless a-h products from both are identical H_3PO_4 NH_4Cl; ninhydrin reacting base vari ous 5,6-dimethylbenziminazoles (I) the nature of which depends upon the conditions of a-h. Moderate a h gives α-N-substituted I (II) stronger a-h gives II and β-N substituted I (III) and still more vigorous a h yields I itself. II contain the basic nucleus represented by I substituted in the 1-position by a phosphorylated side chain (5 or 6 C atoms with several OH groups) III is dephosphorylated II. Both II and III are glucosides. The phosphoryl group is attached to C_2 or C_3 in the sugar residue. It is suggested that B_{12} and B_{12c} are trisubstituted derivatives of phosphoric acid, the base and colored fragment being attached to the I nucleotide (II) through the two free acidic groups in the latter either by ester or amide linkage.

925. EMERSON G., BRINK, N G., HOLLY F W., KONIUSZY F., HEYL, D., and FOLKERS K.: *Vitamin B_{12}. VIII Vitamin B_{12}-like activity of 5,6-dimethylbenzimidazole and tests on related compounds* J Am. Chem. Soc. 72 3084-3085, July 1950.

Two degradation products of vitamin B_{12} are described which show vitamin B_{12}-like growth activity when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder These two products are active at milligram-levels in contrast to vitamin B_{12} which is active at the microgram-level. A third product also has high vitamin B_{12}-like activity while five others have none and one even seemed to depress growth.

Merck Institute for Therapeutic Research, and
Research Laboratories
Merck & Co., Inc.
Rahway, N. J.

926 MAMALIS P., PETROW V., and STURGEON B.: *The chemistry of anti pernicious anaemia factors Part IV Benziminazole glycosides (1) The preparation and properties of some o-nitroanaline glycosides* J Pharm. and Pharmacol. 2 491-502, Aug. 1950.

Glucosidation of o-nitroaniline gives, in general, a mixture of two isomers designated o-nitroaniline glycosides I and II In contrast to results reported by Kuhn and Strobële acetylation of these isomers gives the cor responding o-nitroaniline acetylglycosides I and II. Conversion of the Series II isomer into the Series I isomer has been effected in certain cases by very short contact with hot dilute hydrochloric acid. Reduction of the o-nitroaniline acetylglycosides I and II is accompanied in certain instances, by a merging of the two series and the formation of only one o-phenylenediamine acetylglycoside. Similar results have been obtained and are here recorded, employing m nitro-p-toluidine, 3-nitro-o-4-xylidine and 5-nitro-o-4-xylidine.

927 MAMALIS P., PETROW V., and STURGEON B. *The chemistry of anti pernicious anaemia fac tors. Part IV Benziminazole glycosides. (2) Synthetic routes to the benziminazole 1-D glucopyranosides* J Pharm. and Pharmacol. 2 503-511, Aug. 1950.

By heating o-phenylenediamine-tetraacetyl D-glucoside with ethyl orthoformate, 2-ethoxymethylene-o-phenyl enediamine tetraacetyl D-glucoside has been obtained. The foregoing ethyl *iso*formanilide has been converted into benziminazole-1 β-tetraacetyl D-glucopyranoside by the action of hot dilute hydrochloric acid. The constitu tion assigned to this compound has been confirmed by an alternative synthesis from benziminazole silver and α-acetobromglucose. Benziminazole-1-β D-glucopyranoside has been obtained by hydrolysis of the acetylated glucoside with 6*N* hydrochloric acid and the pyranoside char acter of the lactol ring confirmed by periodate titrations. The foregoing novel synthetic methods have been applied to the preparation of 5-methyl- 4 5-dimethyl- and 5-6-dimethylbenziminazole glucosides. In certain cases re-*action between an o-phenylenediamine acetylglycoside and* ethyl orthoformate has led directly to the formation of the benziminazole acetylglucoside. The benziminazole glucopyranosides prepared in the course of this work showed unexpected stability toward hot 6*N* hydrochloric acid.

928. MAMALIS, P., PETROW V., and STURGEON B. *The chemistry of anti-pernicious anaemia fac tors Part IV Benziminazole glycosides. (3) The preparation of some benziminazole pentosides* J Pharm. and Pharmacol. 2 512-518, Aug. 1950.

New methods elaborated in Part IV (2) for the synthesis of benziminazole glucosides have been extended by the preparation of some benziminazole pentosides. Results obtained employing the "orthoformate route" show that ethoxymethylene-o-phenylenediamine acetylpentosides undergo more facile ring closure than the corresponding glucosides, being readily converted into the benziminazole pentosides by the action of alcoholic picric acid. The pyranoside structures assigned to

foregoing compounds have been confirmed by periodate titrations. Benziminazole pentosides show unexpected stability to acids and are recovered unchanged after heating for 12 hours with 6N hydrochloric acid at 100 C.

929 COOLEY G., ELLIS, B., and PETROW V *The "ninhydrin-reacting" hydrolytic fragment of vitamin B_{12} and 1-aminopropan-2-ol*, J Pharm. and Pharmacol. 2 535, Aug. 1950.

In continuation of earlier work the chromatographic behavior of several amino-alcohols of low molecular weight was studied and it was found that 1-amino-propan-2-ol, a structural isomer of 2-aminopropan-1-ol, and ninhydrin reacting fragment are likewise indistinguishable on paper chromatograms irrigated with a number of different solvent systems. Furthermore, oxidation of 1 amino-propan-2-ol with acid potassium permanganate followed by paper chromatography of the product, gives a yellow spot with the ninhydrin reagent, identical in every respect with that obtained from the ninhydrin-reacting fragment in a similar way Thus, not only is the latter fragment chromatographically inseparable from 1-amino-propan-2-ol, but their respective highly characteristic oxidation products are also inseparable. Independent studies by the Merck group had established the identity of the ninhydrin-reacting fragment with D-1-amino-propan-2-ol by the methods of classical organic chemistry

930. COOLEY G., ELLIS., B., MAMALIS, P., PETROW V., and STURGEON, B.: *The chemistry of anti-pernicious anaemia factors. Part V The interrelationship and structure of the α- β- and γ-components*, J Pharm. and Pharmacol. 2 579-590, Sept. 1950.

931. BEAVEN G H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., and PETROW V *The mode of linkage of component a in vitamin B_{12}*, J Pharm. and Pharmacol. 2 733-735, Oct. 1950.

932. BUCHANAN, J G., JOHNSON A. W., MILLS, J A., and TODD A. R.: *Chemistry of the vitamin B_{12} group. Part I Acid hydrolysis studies. Isolation of a phosphorus-containing degradation product*, J Chem. Soc. 2845-2855, Oct. 1950.

933. BRINK, N. G., and FOLKERS, K. *Vitamin B_{12}. X. 5,6-Dimethylbenzimidazole a degradation product of vitamin B_{12}*, J Am. Chem. Soc. 72 4442 4443, Oct. 1950.

Authors summary: "5,6-Dimethylbenzimidazole has been isolated from an acid hydrolysate of vitamin B_{12}. Its structure has been established by degradation and by synthesis."

Merck & Co., Inc. Rahway, N J

934 BOXER, G. E., and RICKARDS J C. *Chemical determination of vitamin B_{12}. I Determination of 5,6-dimethylbenzimidazole by colorimetric and fluorometric methods*, Arch. Biochem. 29: 75-84, Nov 1950.

A colorimetric and a fluorometric determination of 5,6-dimethylbenzimidazole is described. The colorimetric method employes the reaction of 4,5-dimethyl-o-phenylenediamine with acetylacetone forming the purple sulfate of 2,4,7,8-tetramethyl-1,5-benzodiazepine. The fluorometric method employs the reaction of 4,5-dimethyl-o-phenylenediamine with alloxan forming the fluorescent 6,7 dimethyl-alloxazine. These proposed procedures for the determination of benzimidazoles can be used as a basis for an assay of vitamin B_{12}.

Merck & Co., Inc. Rahway N. J.

935. BEAVEN, G. H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., and PETROW V: *The chemistry of anti-pernicious anaemia factors. VI The mode of combination of component a in vitamin B_{12}*, J Pharm. and Pharmacol. 2 944-955, Dec. 1950.

936. BOXER, G. E., and RICKARDS, J C. *Chemical determination of vitamin B_{12}. II The quantitative isolation and colorimetric determination of millimicrogram quantities of cyanide* Arch. Biochem. 30 372-381, Feb. 1951.

Authors summary "One approach to the specific determination of vitamin B_{12} required a sensitive and specific method for the determination of cyanide. A procedure for the isolation, concentration, and colorimetric determination of 1 part of cyanide in 10^{11} parts of solution with a precision of 1.5 per cent is described. The factors influencing the isolation of millimicrogram quantities of cyanide by aeration, and the separation of hydrogen cyanide from thiocyanate, hydrogen sulfide, and from other volatile substances interfering with the color reaction, are discussed. The conditions for the color reaction for cyanide described by Epstein have been studied and improvements are proposed which increase the sensitivity and precision of the method."

Merck & Co., Inc. Rahway N J

937 BOXER, G E., and RICKARDS, J C. *Chemical determination of vitamin B_{12}. III Methods for the quantitative and specific release of the cyano group of vitamin B_{12}*, Arch. Biochem. 30 382-391, Feb. 1951.

Two procedures for the colorimetric determination of vitamin B_{12} in microgram quantities are described. Both methods are based on the quantitative liberation and colorimetric determination of cyanide complex bound to the cobalt atom of vitamin B_{12}. In one procedure, the cyano group is released by reduction with hypophosphorous acid. In the other procedure the cyano group is released by exposure to visible light. The determination of cobalamins other than vitamin B_{12} is described. It is based on the fact that these substances are converted to vitamin B_{12} by treatment with cyanide. The cyanide thus bound is then determined.

Merck & Co., Inc. Rahway, N J.

938. EMERSON G., HOLLY F W., SHUNK, C H., BRINK, N G., and FOLKERS, K.: *Vitamin B_{12}. XII Vitamin B_{12}-like activity of α- and β-ribazole*, J Am. Chem. Soc. 73 1069-1070, March 1951.

Authors summary: "α Ribazole (1-α-D-ribofuranoside-5,6-dimethylbenzimidazole) a degradation product of vitamin B_{12}, has been found to elicit a marked vitamin

B_{12}-like response when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder It is about one four-hundredth as active as vitamin B_{12} in this test, and on a weight basis may be considered comparable to the activity of riboflavin and pantothenic acid in their respective animal tests. β Ribazole also gave a significant response in the test.

"1 D-Ribityl-5,6-dimethylbenzimidazole appears to have less vitamin B_{12}-like activity than 5,6-dimethylbenzimidazole."

Merck Institute for Therapeutic Research, and Research Laboratories Merck & Co., Inc. Rahway, N J

939 WRIGHT J B. *The chemistry of the benzimidazoles*, Chem. Rev 48 397-541 June 1951.

A review with 769 references.

940. DAVIES, M T., MAMALIS, P., PETROW V., and STURGEON B. *The chemistry of anti pernicious anaemia factors. Part VIII The basicity of some benziminazoles and benziminazole glycosides* J Pharm. and Pharmacol. 3 420-430, July 1951

The pKa values of 5,6-substituted benziminazoles in water and/or aqueous ethanol have been determined. The relationship between basicity and constitution in the benziminazole series is discussed.

941. PFIFFNER, J J., DION H. W., and CALKINS D G. *Further characterization of pseudovitamin B_{12} pigments* Federation Proc. 11 269 March 1952.

"The harvest broth of a rumen anaerobe has yielded 3 pairs of pigments of the pseudo (ψ) series 3 cyanocobalt pigments and the corresponding 3 presumably hydroxo compounds. Two pairs (crystalline) are highly active as growth factors for certain lactobacilli but are inactive in chicks, rats and man (pernicious anemia) The 3rd pair (ca. 30% pure) have no demonstrable biological activity but exhibit ultraviolet absorption properties typical of the series. Two crystalline cyano pigments (ψ-vitamins B_{12} and B_{12b}) when subjected to acid degradation have yielded Dg 1-aminopropanol 2, inorganic phosphate, ammonia, a red cobalt fraction, adenine and a trace of hypoxanthine (paper chromatographic evidence) The hypoxanthine is considered to arise from the deamination of adenine. No indication was obtained for the occurrence of 5,6-dimethylbenzimidazole or its derivatives. The crystalline pigments of the ψ-series apparently contain adenylic acid in place of 5,6-dimethylbenzimidazole-1-α-D-ribofuranosido-phosphate. The presence of adenine instead of 5,6-dimethylbenzimidazole is in keeping with the higher nitrogen and lower carbon content of the ψ-vitamins as compared to vitamin B_{12} itself (ψ-vitamin B_{12} C, 52.06 H, 6.60 N 17.85; Co, 4.40; P 2.31 ψ-Vitamin B_{12b} C, 52.45 H, 6.51; N 18.62 Co, 4.34 P 2.28) Following the cobalamin system of nomenclature for vitamin B_{12}, ψ-vitamin B_{12} may be referred to as cyano-β-cobalamin and ψ-vitamin B_{12b} as cyano-γ-cobalamin."

Parke, Davis & Co. Detroit, Mich.

Analogs

942. KACZKA, E., WOLF D E., and FOLKERS, K.: *Vitamin B_{12}. V Identification of crystalline vitamin B_{12a}* J Am. Chem. Soc. 71 1514-1515, April 1949 (Letter to Editor)

A new and biologically active crystalline compound, designated vitamin B_{12a}, has been obtained by catalytic reaction of vitamin B_{12} with hydrogen. It is somewhat less active than vitamin B_{12}. In a single patient with pernicious anemia, Dr Randolph West, of Columbia University has reported (personal communication) that the parenteral administration of 25 mcg. of vitamin B_{12a} produced about 30 per cent of a maximal hematologic response.

943 PIERCE, J V., PAGE, A. C., JR., STOKSTAD E. L. R., and JUKES, T H. *Crystallization of vitamin B_{12b}* J Am. Chem. Soc. 71 2952, Aug. 1949 (Letter to Editor)

A crystalline fraction has been separated from liver extract by chromatography which has absorption spectrum maxima different from those reported for vitamin B_{12}. Two characteristic pink bands were thus separated and were eluted. Fractional precipitation of the first of these with acetone yielded small rodlike red crystals which contained cobalt and phosphorus, and which were biologically active in the chick assay and in the assay with Lactobacillus leichmannii 313. The term "vitamin B_{12b}" is suggested for this preparation. The second pink fraction had an absorption spectrum which was characteristic of vitamin B_{12}, and was concentrated to yield needle-like crystals that appeared similar to those of vitamin B_{12}.

944 BROCKMAN, J A., JR., PIERCE, J V., STOKSTAD E. L. R., BROQUIST H. P., and JUKES T H.: *Some characteristics of a crystalline compound derived from vitamin B_{12}* J Am. Chem. Soc. 72 1042, Feb. 1950 (Letter to Editor)

The isolation of a red crystalline product from vitamin B_{12} is described. Its biologic activity is similar to that of vitamins B_{12} and B_{12b}. B_{12a}, which has a band in the absorption spectrum which is missing from the absorption spectra of the new product and vitamin B_{12b}, is less effective. The present experimental results indicate that vitamin B_{12a} was produced by hydrogenation of vitamin B_{12}.

Lederle Laboratories Pearl River N Y

945. SMITH, E. L. *Recent work on vitamin B_{12}*, Lancet 1: 353, Feb. 25, 1950 (in Soc. Proc.)

Both microbiological assays and partition chromatography have revealed the presence in liver extract of at least three active substances vitamin B_{12}, an allied vitamin B_{12b}, and thymidine. Also reported was the isolation from Streptomyces griseus of another substance which had proved to be as effective clinically as vitamin B_{12} its chemical and physical properties were close to those of B_{12}. It is referred to as "the unnamed substance." Investigation of the structure of the substance shows the presence of an atom of cobalt in its molecule, a benzimidazole group, and probably a pyrrolic

molecule of ribose phosphorylated at C_2 or C_3. The cobalt-containing moiety is attached to this phosphorus atom; it represents about two-thirds of the molecule, and its structure has not yet been elucidated. In addition, ammonia and two molecules of Dg 1-amino-2-propanol are attached at some point.

The many functions and properties of B_{12} found by other workers are reviewed.

Glaxo Laboratories Ltd.
Greenford, Middlesex, England

959 JACKSON W G., WHITFIELD G B., DEVRIES W H., NELSON H., and EVANS J S. *The isolation of vitamin B_{12b} from neomycin fermentations*, J Am. Chem. Soc. 73 337-341 Jan. 1951.

Vitamin B_{12b} has been isolated from liver from aureomycin fermentations and from streptomycin fermentations. The present paper describes an isolation process by which it may be obtained as a co-product of neomycin produced by fermentation. The neomycin fermentation medium is acidified and the vitamin is freed from the mycelium by heating, and adsorbed on carbon. The additional steps by which the isolation is completed and the product purified are described. The final crystalline product occurs as dark red needles.

Crystalline vitamins B_{12} and B_{12b} are compared by means of three microbiologic assays. The results are in line with the observations of others on the instability of B_{12b} in the presence of certain reducing agents and assay media constituents, which may account for apparent differences in the microbiologic assay of these vitamins. The ultraviolet, visible and infrared absorption curves of the two vitamins are presented.

The Upjohn Company
Kalamazoo, Mich.

960. PENNINGTON R. J *A heat-labile vitamin B_{12} complex in faeces*, Biochem. J 48 xviii, Jan. 1951 (in Proc. Biochem. Soc.)

961. COOLEY G., ELLIS, V., PETROW V., BEAVEN G H., HOLIDAY E. R., and JOHNSON E. A. *The chemistry of anti pernicious anaemia factors. Part VII Some transformations of vitamin B_{12b}*. J Pharm. and Pharmacol. 3 271 285, May 1951.

The bathochromic shift observed in the spectrum of vitamin B_{12b} in passing from pH 12 to pH 2 is interpreted as due to aquation of hydroxocobalamin with formation of an aquocobalamin cation. The reaction mechanism underlying the stepwise conversion of vitamin B_{12} into cyanocobalamin and dicyanocobalamin is discussed. Ammonia cobalichrome, structurally analogous to ammonia haemochrome, has been prepared from vitamin B_{12a} and its conversion into cyanocobalamin effected. Evidence is presented for the existence of histidine cobalichrome, likewise converted into cyanocobalamin by cyanide. A hypothetical scheme for a possible role of vitamin B_{12} in metabolic processes is presented.

962. BUHS, R. P., NEWSTEAD E. G., and TRENNER, N R.: *An analog of Vitamin B_{12}*, Science 113 625-626, June 1, 1951.

The chemistry of the vitamin B_{12} cobalt complex is discussed. It is stated that the cyanide ion (CN^-) may be replaced by other ions or uncharged groups, thus forming analogs of vitamin B_{12}.

In the present experiment the authors have prepared the thiocyanate analog of vitamin B_{12} by reacting vitamin B_{12a} with potassium thiocyanate. This analog, which crystallizes in purple-red needles, has full biologic activity when compared with vitamins B_{12} and B_{12a} by several methods of assay Acute toxicity tests on mice failed to reveal any detectable toxicity at the equivalent level of 3.2 mg. of the analog for a 70-Kg. man, and preliminary clinical reports have indicated that the thiocyanate analog of vitamin B_{12} is fully active for pernicious anemia.

Chemical and physical properties of the new analog are compared with those of vitamins B_{12} and B_{12a}. The thiocyanate analog is not compatible with ascorbic acid at a level of 20 mcg./cc. of the analog to 20 mg./cc. of ascorbic acid. Vitamin B_{12a} also reacts with ascorbic acid. Vitamin B_{12} does not.

Merck & Co., Inc.
Rahway N J

963 KACZKA, E. A., WOLF D E., KUEHL, F A., JR., and FOLKERS, K.: *Vitamin B_{12}. XVI Modifications of cyanocobalamin*, J Am. Chem. Soc. 73 3569-3573, Aug. 1951.

Vitamin B_{12} (cyano-cobalamin) reacts with sulfurous acid to yield sulfato-cobalamin and with hydrogen sulfide to give a sulfur-containing cobalamin. Vitamin B_{12a} (hydroxo-cobalamin) reacts with chloride, bromide, sulfate, cyanate and nitrite ions to give chloro-, and bromo- sulfato- cyanato- and nitro-cobalamin. The cobalamin derivatives are converted to vitamin B_{12} (cyano-cobalamin) by reaction with cyanide ions. An interpretation of the formation of vitamin B_{12}-like compounds from vitamin B_{12} is discussed.

964 COOLEY G., ELLIS B., PETROW, V., BEAVEN G. H., HOLIDAY E. R., and JOHNSON E. A. *Some observations on the relationship between vitamins B_{12a} and B_{12b}*. J Pharm. and Pharmacol. 3 607-608, Sept. 1951.

The contradictory views of various authors on the relationship of vitamins B_{12a} and B_{12b} are reviewed and discussed. No conclusive proof is seen that vitamins B_{12a} and B_{12b} are identical in the solid state.

965. EMERSON G., and FOLKERS K.: *Water soluble vitamins. Vitamin B_{12} or cyanocobalamin and related compounds*, Ann. Rev Biochem. 20 559-574, 1951.

966. LEWIS, U J., TAPPAN D V., and ELVEHJEM, C. A.: *A new and biologically different form of vitamin B_{12}*, J Biol. Chem. 194 539-548, Feb. 1952 (abstr Blood 7 759 July 1952)

"Since the isolation of vitamin B_{12} in 1948, numerous forms of the vitamin have been reported. Although B_{12a} and B_{12b} are identical, B_{12c} is a more highly oxygenated form. All of these seem to have the same biologic activity when measured by animal growth responses. This is a

report on characteristics of a material isolated from the feces of rats fed a corn-soybean meal diet supplemented with cobalt sulfate. Although it has growth-promoting properties for L. leichmannii and chicks, it is inactive in this respect for the rat. The chromatographic pattern and absorption spectrum of the material are not identical with those of vitamin B_{12}."

University of Wisconsin
Madison, Wis.

967 FORD, J. E., HOLDSWORTH, E. S., KON, S. K., and PORTER, J. W. G.: *Occurrence of the various vitamin B_{12} active compounds*. Nature 171: 150-151, 1953 (abstr. Blood 8: 486-487, May 1953)

"Many of the substances with activity similar to that of vitamin B_{12} are probably not pure substances. In this paper are discussed the possible causes of activity of pseudo-vitamins B_{12} and B_{12b}, factor A, vitamin B_{12m}, B_{12f}, factor WII, factor B and factor C.

"The vitamin B_{12} activity for micro-organisms of extracts of gut contents and feces is contributed in varying proportions by five substances, viz. factors A, B, and C, pseudo-vitamin B_{12}, and vitamin B_{12} itself. In natural materials these compounds may exist in both free and combined forms.

"As these vitamin B_{12} active compounds are formed by microbial synthesis in the rumen or large intestine or both, it is probable that the relative amounts of each that appear in gut contents depend on the composition of the microbial flora. This will vary with the nature of the diet."

University of Reading
Reading, England

U. S. PHARMACOPEIA — U. S. PATENT OFFICE

968. U. S. P. REVISION COMMITTEE: *Vitamin B_{12} admitted to U. S. P. XIII*. J. Am. Pharm. A. (Pract. Pharm. Ed.) 10: 544, Sept. 1949

Crystalline vitamin B_{12}, crystalline vitamin B_{12} injection, and vitamin B_{12} concentrate are being admitted to the U. S. P. XIII by Interim Revision. Recommendations on claims for vitamin B_{12} are quoted.

969 UNITED STATES PHARMACOPEIA: *U.S.P. Committee by interim revision admits vitamin B_{12} to U.S.P. XIII*. Merck Rep. 59: 7, Jan. 1950.

Because of the immediate importance of vitamin B_{12} in the treatment of pernicious anemia, the U.S.P. Revision Committee is admitting to U.S.P. XIII by interim revision, rather than waiting for publication of U.S.P. XIV, the following items: Crystalline Vitamin B_{12}, Crystalline Vitamin B_{12} Injection, Vitamin B_{12} Concentrate. Since a reliable microbiological assay for the vitamin B_{12} content of commercial anti-anemia preparations has not yet been standardized, U.S.P. units are to be used for the present.

970. COUNCIL ON PHARMACY AND CHEMISTRY: *Vitamin B_{12}-U.S.P.—Cobione (Merck)*. J.A.M.A. 143: 1342-1343, Aug. 12, 1950.

Cobione has been accepted by the Council. Crystalline vitamin B_{12} has hemopoietic activity apparently identical with that of the antianemia factor of liver. It has, however, not been established as the complete or essential counterpart of this factor. Studies indicate that vitamin B_{12} is clinically efficacious in the treatment of pernicious anemia, with or without neurologic complications, tropical and nontropical sprue, nutritional macrocytic anemia due to vitamin B_{12} deficiency and in certain cases of megaloblastic anemia of infancy. Cobione is particularly useful for treating patients who are sensitive to liver extract. The Council is not as yet able to make a statement as to the effectiveness of crystalline vitamin B_{12} as compared with liver extract in the *treatment of spinal* cord lesions associated with pernicious anemia, because the follow-up period on such patients has not been sufficiently long. The effects of crystalline vitamin B_{12}, thus far observed, however, have been as good as those of liver extract.

In man, no toxic reactions due to Cobione have been reported; the drug is also not toxic to animals when given orally or subcutaneously. The minimum effective dosage of crystalline vitamin B_{12} is believed to be about 1 mcg. a day, usually given in multiples of this amount at longer intervals, for instance 15 mcg. every two weeks. One microgram of Cobione is estimated to be about equal biologically to one U.S.P. "injectable" unit of liver extract. Recommended parenteral Cobione dosages are as follows: In uncomplicated pernicious anemia, 15 mcg. once or twice a week until remission occurs, then a maintenance dose of 15 mcg. every other week. In pernicious anemia with neurologic complications, 15 to 30 mcg. once or twice weekly followed by a maintenance dose of 15 mcg. every other week. In sprue, 15 to 30 mcg. once or twice weekly until remission occurs, followed by 15 mcg. once a week to prevent relapse. In megaloblastic anemia of infancy, the dosage is the same as for sprue, but if remission is not prompt, folic acid should be given instead of vitamin B_{12}. In nutritional macrocytic anemia, a single dose of 15 mcg. of Cobione will usually produce a favorable initial response, but this dose must sometimes be repeated at two-week intervals to prevent relapse. Satisfactory responses are obtained with oral Cobione, when high doses are given. Most patients respond slowly to oral doses of vitamin B_{12} which are by weight 30 to 60 times the size of those used in parenteral therapy. This amount can be reduced by giving the vitamin along with normal gastric juice.

971 WOLF, F. J.: *Process for recovering vitamin B_{12}*. *U. S. 2,530,416*. Official Gaz. U.S. Pat. Off. 640: 779, Nov. 21, 1950.

"In a process for recovering vitamin B_{12} from a starting material selected from the class consisting of fermentation broths obtained by the propagation of a vitamin B_{12} producing microorganism in a suitable nutrient medium and concentrates thereof, the step that comprises treating the starting material with a substa furnishing cyanide ions, thereby obtaining a re mixture containing an increased amount of vita

972. RICKES, E. L., and WOOD T. R. *Vitamin B_{12}* *U S 2,563,794* Official Gaz. U S. Pat. Off. 649 302, Aug. 7 1951.

"The compound vitamin B_{12}, an organic substance containing cobalt, together with carbon, nitrogen, hydrogen, oxygen and phosphorus, said compound being a red crystalline substance soluble in water methyl and ethyl alcohol and phenol, and insoluble in acetone, ether and chloroform, and exhibiting strong absorption maxima at about 2780Å, 3610Å and 5500Å, and an L.L.D. activity of about 11,000,000 L.L.D. units per milligram."

973. THE CHEMICAL WORLD THIS WEEK: *Method for producing vitamin B_{12}*, Chem. & Engin. News 30 1746, April 28, 1952.

U.S.D.A. patent 2,561,364. "Vitamin B_{12} is produced by cultivating Flavobacterium devorans in a medium containing assimilable carbon source and an assimilable nitrogen source. The product is recovered as a vitamin concentrate by evaporating the culture liquor after fermentation is complete."

974 PATTERSON A. M.: *Amino acids carotenoids, vitamins*, Chem. & Engin. News 30 104, Jan. 7 1951.

The following names have been adopted by the IUPAC Commission of the Nomenclature of Biological Chemistry:

B_1 (aneurin, thiamine)	thiamine
B_2 (riboflavin)	riboflavin
B_{12} (collectively)	cobalamin
B_{12} (pure)	cyanocobalamin
B_{12a}	hydroxocobalamin
B_{12c}	nitrosocobalamin
E	α,β, and γ-tocopherol
PP (niacinamide, nicotinamide)	nicotinamide

The names p-aminobenzoic acid, ascorbic acid, biotin, choline, and pantothenic acid are confirmed.

MICROBIOLOGIC ASSAYS

975. SHORB, M. S. *Activity of vitamin B_{12} for the growth of* Lactobacillus lactis, Science 107 397 398, April 16, 1948.

The minute amount of the new crystalline compound isolated from liver vitamin B_{12}, which is required by Lactobacillus lactis Dorner for growth places it among the most potent microbiologically active compounds. Its extremely high potency, 11,000,000 units per mg. with a 23-hour growth period and 17,000,000 units per mg. with one of 42 hours, compared with an assigned potency of 1,000 units per mg. for the arbitrary liver concentrate standard used in the LLD factor assays, suggests that it is wholly or partially responsible for the LLD growth activity observed with liver extracts. The above standard was free from the other factor TJ also required by L. lactis, at levels as high as 500 micrograms per tube. Vitamin B_{12} has little or no effect on the growth of L. casei, L. fermenti, L. arabinosus, Streptococcus fecalis R, and Escherichia coli. These organisms also grow readily without supplementation with TJ but they have been found to synthesize this factor

Studies of source materials reported to contain unidentified factors for chicken nutrition revealed that LLD factor activity occurs in fairly high amounts, in approximately decreasing order in a papain digest of acid precipitate from cow manure, the acid precipitate from cow manure, fish meal, pancreatin, papain, egg white, and egg yolk, and in lower amounts in alcoholic extract of whey potassium permanganate–oxidized alcoholic extract of whey soybean oil meal, gelatin, zein, and Mylase P enzyme. TJ factor activity is also found, in approximately decreasing order in the papain digest of acid precipitate from cow manure, the acid precipitate of cow manure, egg yolk, papain, and pancreatin, but in much lower amounts in fish meal, alcoholic extract of whey soybean oil meal, crude casein, egg white, zein, Mylase P enzyme, potassium permanganate–oxidized alcoholic extract of whey and gelatin. The distribution of LLD and TJ factor activities in these materials suggests that they may be involved in chicken nutrition.

976. SHORB, M. S., and BRIGGS, G. M. *The effect of dissociation in* Lactobacillus lactis *cultures on the requirement for vitamin B_{12}*, J Biol. Chem. 176 1463-1464, Dec. 1948 (Letter to Editor)

Although Lactobacillus lactis Dorner is stable in its requirement for both vitamin B_{12} (LLD factor) and the tomato juice (TJ) factor when cultured on a tomato-juice, yeast-extract, skim-milk medium, the culture undergoes dissociation when serial transfers are made. The medium used in the assay of the LLD factor and in connection with the isolation of vitamin B_{12} is that of Baumgarten *et al.* (*J Am. Chem. Soc.* 66 1607 1944) modified by the addition of all the B vitamins at tenfold concentration, except biotin and pteroylglutamic acid, which are added at 0.05 microgram per cc. Adenine, guanine, uracil, and xanthine are also added, together with 0.5 cc. of TJ factor This assay works well with the stabilized culture and purified liver extracts or crystalline vitamin B_{12} but erratic results may be obtained with the dissociating culture or with some crude materials because of inhibitory substances. This inhibition is partly due to high concentrations of folic acid. High levels of serine, para-aminobenzoic acid, xanthine, and certain salts also inhibit growth. Culture filtrates of Lactobacillus casei, Streptococcus fecalis R, and Escherichia coli and fresh chicken droppings have low vitamin B_{12} activity

977 HOFFMANN C. E., STOKSTAD E. L. R., FRANKLIN, A. L., and JUKES, T H.: *Response of* Lactobacillus leichmannii 313 *to the anti-pernicious anemia factor* J Biol. Chem. 176 1465-1466, Dec. 1948 (Letter to Editor)

Thymidine has a growth-stimulating effect upon Lactobacillus lactis Dorner under conditions in which the growth is also promoted by vitamin B_{12}. Thymidine also promotes the growth of L. leichmannii 313. The effect of a sample of crystalline anti-pernicious anemia (APA) factor was tested with this organism. The results indicate that L. leichmannii 313 may be a sensitive test organism for the APA factor and that liver extract produces a similar response.

978. SKEGGS, H R., HUFF J W., WRIGHT L. D., and BOSSHARDT D K.: *The use of* Lactobacillus leichmannii *in the microbiological assay of the "animal protein factor"* J Biol. Chem. 176 1459-1460, Dec. 1948 (Letter to Editor)

Lactobacillus leichmannii has given very satisfactory results as an assay organism for the "animal protein factor" Recent reports from the Merck laboratories have indicated that the "animal protein factor" is identical with or closely related to vitamin B_{12}. If this is true, it is probable that L. leichmannii may be the organism of choice for the microbiologic assay of vitamin B_{12}. Lactobacillus lactis is currently used for the assay of vitamin B_{12}, but this organism has very complicated growth requirements, which makes its use difficult. Both L. leichmannii and L. lactis respond to purified liver preparations (known to be rich in "animal protein factor") and both organisms are stimulated in growth by thymidine.

979 HUTNER, S. H., PROVASOLI L., STOKSTAD E. L. R., HOFFMANN C. E., BELT M., FRANKLIN A. L., and JUKES, T H *Assay of anti-pernicious anemia factor with* Euglena, Proc. Soc. Exper Biol. & Med. 70 118-120, Jan. 1949

The algal flagellate Euglena gracilis var bacillaris exhibits a quantitative growth response to crystalline anti-pernicious anemia factor (vitamin B_{12}) when grown in a basal purified culture medium. Thymidine is inactive, although it is effective for lactobacilli. This suggests that biochemical generalizations regarding the functions of anti-pernicious anemia factor should not be made solely on the basis of observations with lactobacilli.

980. SHAW G. E.: *Liver extracts*, Lancet 1 239-240 Feb. 5, 1949 (in Letters to the Editor)

The writer thinks it probable that some modification of the Lactobacillus lactis Dorner assay of vitamin B_{12} will eventually provide a unitage system for describing the anti-pernicious anemia potency of liver extracts. A technic for this assay has been evolved in the Evans Biological Institute, and an alternative cup method for the determination of LLD growth factors has recently been announced by Cuthbertson. If a liver extract contained only clinically active vitamin B_{12}, the total LLD activity would measure the clinical potency Some growth factors for L. lactis, however have no clinical activity against anemia. It is suggested that by means of paper chromatography the amount of each factor present in a particular extract might be determined. If a number of liver extracts with different spectra were assayed clinically it would probably be possible to translate vitamin B_{12} microbiologic activity into clinical potency Such experiments are under way

981. SMITH, E. L., and CUTHBERTSON, W F J: *Liver extracts*, Lancet 1 325, Feb. 19 1949

Microbiological assay has been used to supplement, though in no way to replace, clinical tests. It has been found that liver extracts contain four substances active in the microbiological assay of which only two have been proved clinically active.

It is hoped that clinical tests may eventually be replaced by combination of chromatography (paper strip?) and microbiological assay but comparison of the two methods on a large number of batches can alone provide information to justify such a procedure.

982. CAPPS B F., HOBBS N L., and FOX, S H: *A method for the microbiological assay of vitamin B_{12}*, J Biol. Chem. 178 517-518, March 1949 (Letter to Editor)

In the microbiologic assay of vitamin B_{12}, Lactobacillus leichmannii may be preferable to Lactobacillus lactis Dorner as the test organism, since it shows less tendency to dissociate. The standard (purified vitamin B_{12} Merck) and test materials are diluted to 0.02 mcg. per cc. in distilled water The test organism responds best within the range of 0.01 to 0.10 mcg. per tube levels. Duplicate and triplicate tubes on each level give growth response in good agreement turbidimetrically Tubes containing only distilled water and the basal medium without B_{12} produce no growth. Further studies are under way to determine the relative merits of this method of assay of natural materials containing minute amounts of vitamin B_{12} and a similar method in which Lactobacillus lactis Dorner is used as the test organism.

983 STOKSTAD E. L. R., DORNBUSH, A. C., FRANKLIN A. L., HOFFMANN C. E., HUTCHINGS B. L., and JUKES T H.: *Microbiological assay of vitamin B_{12} by* Lactobacillus leichmannii Federation Proc. 8 257 March 1949

Assay of vitamin B_{12} by means of the growth response of Lactobacillus leichmannii is discussed, particularly in regard to the action of thioglycollic acid and other substances in protecting the vitamin from destruction during autoclaving of the samples with the medium.

984 GREENE, R. D., BROOKS, A. J., and McCORMACK, R. B.: *Some conditions which affect the assay of vitamin B_{12} with Lactobacillus lactis Dorner* J Biol. Chem. 178 999-1000, April 1949

The assay of vitamin B_{12} with Lactobacillus lactis Dorner is influenced by the degree of aerobiosis in the broth cultures. Three minutes autoclaving in 23 x 150 mm. tubes gave consistent assays. The growth promoting activity of B_{12} for L. lactis and L. Leichmannii was completely inhibited by folic acid and almost completely by aminopterin, while teropterin and N^{10} methypteroic acid were not antagonistic.

985 SHAW G E. Lactobacillus lactis Dorner *for the assay of vitamin B_{12}*, Nature 164 186-187 July 30, 1949 (Letter to Editor)

A technic is described in detail for obtaining consistent results in Lactobacillus lactis Dorner assays of vitamin B_{12}, which previously have shown wide variations. This organism shows a remarkable proneness to change its morphology under different cultural conditions, and these changes alter its growth characteristics and sensitivity to vitamin B_{12}. A uniform degree of insensitivity is difficult to insure, but in the Evans Biological Institute the organism has been maintained in a co

condition of high sensitivity from a single colony isolation through more than 150 daily subcultures on agar containing 1% "Evans Bacteriological Peptone," 0.1% "Evans Hepamino," 0.1% "Tween 80," and either 5% tomato juice or 0.01% potato extract fraction.

986. KODITSCHEK, L. K., HENDLIN D., and WOODRUFF H. B. *Investigations on the nutrition of Lactobacillus lactis Dorner* J Biol. Chem. 179 1093-1102, July 1949

An unidentified growth factor LLD, which is required by Lactobacillus lactis Dorner has been reported by Shorb. Efforts to utilize L. lactis Dorner for assay of the LLD growth factor have shown that the requirement is affected by various environmental factors, which are described.

987 CASWELL, M. C., KODITSCHEK, L. K., and HENDLIN D : *The microbiological estimation of Lactobacillus lactis Dorner activity with vitamin B_{12} as a standard*, J Biol. Chem. 180 125-131 Aug 1949

The authors describe in detail the method of assay for vitamin B_{12} used in the Merck laboratories, where this vitamin was first isolated. It is based upon the response of Lactobacillus lactis Dorner variant 6a to vitamin B_{12}, as measured by the lactic acid formed from the dissimilation of glucose. The specific effect of oxygen tension on the assay and means of eliminating such effect are discussed. In an addendum, the authors state that a threefold increase in precision has been obtained in the assay by substituting L. lactis Dorner ATCC 8000 for the 6a variant. A precision range of ± 20 per cent can be reached with only six replicates.

988. SHAW G E.: *Notes on microbiological assays using Lactobacillus lactis Dorner* J Pharm. and Pharmacol. 1 695-700 Oct. 1949

A basal medium (consisting of H_2SO_4 hydrolyzed casein, tryptophane, vitamin B complex, Tween 80 and specially prepared TJ Factor) and a procedure for the microbiological assay of vitamin B_{12} are described, which have given reproducible results over a long period.

989 PEELER, H. T., YACOWITZ, H., and NORRIS, L. C.: *A microbiological assay for vitamin B_{12} using Lactobacillus leichmannii*, Proc. Soc. Exper Biol. & Med. 72 515-521 Nov 1 1949

A microbiologic assay for vitamin B_{12} using Lactobacillus leichmannii has been developed. The assay medium contains crystalline amino acids and the nitrogen source and adsorbed tomato juice filtrate as a source of unidentified factors. A high level of cysteine and ferrous sulfate in the medium keeps it in the necessary reduced state. This method appears to be specific for vitamin B_{12}. A tabulation of the results of its application to various food substances showed the highest content of vitamin B_{12} in Wilson s liver L (399 mcg./Gm.) red fish meal (111 mcg./Gm.) and crude casein (104 mcg./Gm.) and the lowest in wheat (7 mcg./Gm.) and dried brewers yeast (8 mcg./Gm.)

Cornell University
Ithaca, N Y

990. FOSTER, J C., LALLY J A., and WOODRUFF H. B.: *Cup assay with vitamin B_{12} as a standard*, Science 110 507-509 Nov 11, 1949

The microbiologic estimation of LLD (Lactobacillus lactis Dorner) activity by means of Lactobacillus lactis or Lactobacillus leichmannii, using vitamin B_{12} as a standard, is influenced by degree of aeration, by oxidation-reduction potential, and by accumulation of peroxides. These factors are difficult to control in the titrimetric assay procedure. The cup assay method proposed by Heatley for the estimation of penicillin, which has been used in assays for biotin, thiamine, and riboflavin, allows more rigid control of influential factors and hence gives much more precise results. Also, it is possible by this method, by use of abnormal salt concentrations, to eliminate the diffuse growth response of a test culture to desoxyribonucleic acid or its constituent nucleosides, whereas the Shorb titrimetric assay shows LLD activity for these substances. Cup and titrimetric assays show somewhat different responses to modified substances, such as vitamin B_{12a}, when assayed against a vitamin B_{12} standard. Impure materials, containing various LLD active substances, do not necessarily yield equal results by both methods. Shorb s liver concentrate standard, assigned a value of 1,000 LLD units per mg. by titrimetric assay contained 1,120 units per mg. by cup assay with crystalline vitamin B_{12} as a standard.

Merck & Co., Inc.
Rahway N J

991 HOFFMANN C. E., STOKSTAD E. L. R., HUTCHINGS B. L., DORNBUSH, A. C., and JUKES, T H. *The microbiological assay of vitamin B_{12} with Lactobacillus leichmannii*, J Biol. Chem. 181 635-644, Dec. 1949

An assay method for B_{12} using one of two strains of Lactobacillus leichmannii is described. One strain grows more rapidly than the other thus shortening the assay period, but both give satisfactory results. To prevent destruction of B_{12} when the assay tubes are autoclaved it is necessary to add a reducing agent. Alternatively, sterile assay samples can be added to the autoclaved medium. A growth factor was produced in the medium during autoclaving unless sucrose was substituted for glucose. The growth factor was not needed if thioglycolic acid or an extract of asparagus was included in the medium. The effect of desoxyribosides, which replace vitamin B_{12} for the growth of L. leichmannii, was measured by assaying the samples before and after alkaline hydrolysis which destroyed vitamin B_{12}.

992. WHITE, W F., MOTE, J R., and HAYS E. E.: *A preliminary study of the relationship between Lactobacillus lactis Dorner response and clinical antipernicious anemia activity of liver extracts*, Blood J Hematol. 4 1357 1360 Dec. 1949

Armour Laboratories
Chicago, Ill.

993 KOCHER, V : *Zur Technik der mikrobiologischen Bestimmung von Vitamin B_{12} mit Lactobacillus lactis (The microbiologic determination of vitamin B_{12} with Lactobacillus lactis)* Intern. Z. Vitaminforsch. 20 369-374, 1949

In a synthetic medium containing tomato juice and "peptone witte" (I) L. lactis 1175 requires vitamin B_{12} for growth under aerobic, but not under anaerobic, conditions. Without the addition of I B_{12} is required both under anaerobic and aerobic conditions. Vitamin C may substitute for B_{12} in both cases.

994 SHAW G E *Vitamin B_{12} with some notes on Lactobacillus lactis Dorner* Australasian J Pharm 30 97 1949

A review of the growth factor vitamin B_{12}.

995 CUTHBERTSON W F J: *Estimation of the anti pernicious anaemia factor* Biochem. J 44 No. 2 v 1949 (in Soc. Proc.)

The author has been unable to obtain a response to anti-pernicious anemia factor (APAF) using the method of Shorb and Briggs for B_{12} assay (*J Biol. Chem.* 176 1463, 1948 [Abstr 976]) since the medium used by these investigators appears to be deficient in growth factors for the strain of Lactobacillus lactis with which he has been working. He describes a cup plate assay method which is adaptable to the determination of APAF activity and to the detection of other members of the B_{12} group of microbiologic growth factors in purified liver extracts. The medium employed is essentially that of Roberts and Snell (*J Biol. Chem.* 163 499 1946) with the addition of tomato juice, Tween 80, and 2 per cent agar Under the conditions of test, the diameters of the zones of microbiologic activity are proportional to the logarithms of the APAF concentrations over the range of 0.02 to 0.05 γ of crystalline APAF per cc. Both of these factors contribute to microbiologic activity and unless the ratio of clinical to microbiologic activity is the same for both, this test alone will not predict exactly the clinical potency of liver extracts. Although the method is relatively insensitive and somewhat susceptible to interference by other members of the B_{12} group, preservatives, and antibiotics, this is usually easy to detect and the procedure has the advantage of speed and simplicity

Glaxo Laboratories Ltd.
Greenford Middlesex, England

996. THOMPSON H. T., DIETRICH, L. S., and ELVEHJEM, C. A. *The use of Lactobacillus leichmannii in the estimation of vitamin B_{12} activity* J Biol. Chem. 184 175-180, May 1950.

Authors summary: "A microbiological assay for vitamin B_{12} activity for Lactobacillus leichmannii ATCC 4797 has been developed. *Satisfactory standard curves,* low blanks, and satisfactory recovery values were obtained.

"A method for the release of vitamin B_{12} activity from animal material has been described.

"The vitamin B_{12} activity of various animal materials has been presented."

University of Wisconsin
Madison, Wis.

997 SKEGGS, H. R., NEPPLE, H. M., VALENTIK K. A., HUFF J W., and WRIGHT L. D: *Observations on the use of Lactobacillus leichmannii 4797 in the microbiological assay of vitamin B_{12}* J Biol. Chem. 184 211 221 May 1950.

An assay procedure for vitamin B_{12} employing Lactobacillus leichmannii 4797 is described. This procedure has been found reliable and reproducible. Various problems encountered in the use of this method are discussed.

Sharp and Dohme Inc.
Glenolden, Pa.

998. BESSEL, C. J., HARRISON E., and LEES K. A.: *Assay of vitamin B_{12} with a mutant of Escherichia coli*, Chem. & Ind., p. 561 July 15, 1950.

Using the cup-plate procedure previously described for the assay of aneurin and riboflavin, and also of vitamin B_{12} with Lactobacillus lactis Dorner an assay method for B_{12} with a mutant of E. coli, requiring B_{12} for growth, has been developed. The method is less sensitive than the tube method, involving the use of Lactobacillus leichmannii 313, but more sensitive than the plate methods with either L. leichmannii or L. lactis Dorner

999 LOY H. W., JR., HAGGERTY J F., and KLINE, O L. *A cause of variation in the microbiological assay for vitamin B_{12}*, Arch. Biochem. 29 451, Dec. 1950 (in Letters to Editor)

The importance of adequate rinsing of glassware used in microbiological assays for vitamin B_{12} is discussed. The presence of small amounts of detergents on glassware causes inhibition of the growth of the test organisms, giving incorrect B_{12} values.

Food and Drug Administration
Washington 25, D. C.

1000. HENDLIN D., and SOARS, M. H. *Comparative microbiological studies with vitamin B_{12} and B_{12a}*, J Biol. Chem. 188 603-610 Feb. 1951.

Authors summary "The microbiological activities of vitamin B_{12} and B_{12a} depend markedly upon the assay organism and technique employed.

"In the Lactobacillus leichmannii assay the growth-promoting effect of vitamin B_{12a} is destroyed by autoclaving with the medium vitamin B_{12} is unaffected. This destruction is prevented by the addition of suitable quantities of thioglycolic or ascorbic acid. Since many liver samples show a comparable loss of activity upon autoclaving with the L. leichmannii assay medium, it appears that they contain appreciable quantities of vitamin B_{12a} or B_{12a} like substances.

"In the case of Lactobacillus lactis there appears to be no loss of microbiological activity of vitamin B_{12} or B_{12a} during autoclaving with the assay medium. The activity of both vitamins was increased by autoclaving and the addition of reducing substances. In addition these conditions lead to an increase in the potency of vitamin B_{12a} and so it reaches the biological activity of vitamin B_{12}.

"No apparent differences in microbiological activity were observed between vitamin B_{12a} and a substance described in the literature as vitamin B_{12b}."

Merck & Co., Inc.
Rahway N J

1001 LARKIN F E., and STUCKEY R. E.: *Some observations in the cup-plate assay of vitamin B_{12} using Lactobacillus lactis Dorner 10697* , 761 150-154 March 1951

The cup-plate assay with L. lactis Dorner 10697 is more reliable than tube tests with various organisms. The effect of the pH and cresol concentrations was studied. The assay was found to be particularly useful in determining the stability of samples of vitamin B_{12} of differing purities. With other vitamin B_{12} preparations the microbiological assay was always interpreted in conjunction with organic cobalt determinations. Assays of vitamin B_{12} concentrates obtained from Streptomyces fermentation liquors gave results that were difficult to interpret, and it was considered necessary to use the assay in conjunction with a chromatographic procedure, although some difficulty was experienced in getting a quantitative elution of the small amounts present on the chromatogram.

1002. CUTHBERTSON, W F J., PEGLER, H. F., and LLOYD J T *The assay of vitamin B_{12}. Part III Microbiological estimation with Lactobacillus lactis Dorner by the plate method*, Analyst 76 133-140 March 1951.

The cup plate method for the rapid microbiological assay of aneurine and riboflavine has been found applicable to the estimation of vitamin B_{12} with a suitable strain of Lactobacillus lactis Dorner Factors affecting the "zone" response of this organism to vitamin B_{12} have been investigated they include the effects of vitamin B_{12a} and the deoxyribosides, times of incubation and standing before incubation, range of concentrations of the vitamin and density of the inoculum. The standardized assay permits rapid estimates of potency over a range sufficiently wide and with fiducial limits sufficiently narrow for routine purposes, without the use of an inconveniently large number of assay plates.

1003 EMERY W B., LEES, K. A., and TOOTILL, J P R.: *The assay of vitamin B_{12}. Part IV The microbiological estimation with Lactobacillus leichmannii 313 by the turbidimetric procedure* Analyst 76 141 146, March 1951.

Details are presented of a microbiological tube assay for vitamin B_{12} with Lactobacillus leichmannii 313 as test organism. Statistical analyses of a (3 + 3) assay and a standard response curve are given and show that the method is sufficiently sensitive and accurate for routine use.

1004 BROQUIST H. P., STOKSTAD, E. L. R., and JUKES, T H.: *Further observations on the microbiological assay for vitamin B_{12} using Lactobacillus leichmannii*, Proc. Soc. Exper Biol. & Med. 76 806-811, April 1951.

In an attempt to compare vitamin B_{12b} with vitamin B_{12}, the technic of assaying the two vitamins by aseptic addition to the medium was reinvestigated. It was repeatedly found that the growth response of Lactobacillus leichmannii was less when vitamin B_{12} was added aseptically to the medium than when this vitamin was autoclaved in the medium. In contrast, liver extract, vitamin B_{12b}, B_{12a} and amino-cobalamin (Compound AC2, Drug Houses) gave essentially the same growth when added to the culture medium aseptically autoclaved. As a consequence, erroneously hig were obtained when these samples were assayed against vitamin B_{12} using aseptic technic.

By means of paper strip chromatography it was demonstrated that when vitamin B_{12} is autoclaved with thiomalic acid, the vitamin is transformed into a new compound which is distinct from vitamin B_{12a} and is more potent microbiologically when the compounds are compared by aseptic assay technic.

The significance of these results in the interpretation of the microbiologic assay of the vitamin B_{12} content of natural materials when vitamin B_{12} is used as a standard is briefly discussed.

An addendum states that during the progress of this work the authors received a copy of a paper by H. W Long and O L. Kline (in press) in which it was postulated that both vitamin B_{12} and vitamin B_{12b} are converted to a form of vitamin B_{12} more readily utilizable by L. leichmannii after heating in the basal medium.

*Lederle Laboratories
Pearl River N Y*

1005. CUTHBERTSON W F J., and LLOYD J T *The assay of vitamin B_{12}. I Factors affecting the response of Lactobacillus lactis Dorner* ATCC *8000 to vitamin B_{12}*, J Gen. Microb. 5 416-420, May 1951

1006. SOARS, M. H., and HENDLIN, D *The use of potassium cyanide in the Lactobacillus leichmannii assay for vitamin B_{12}*, J Bact. 62 15-17 July 1951

1007 COOPERMAN J M., DRUCKER, R., and TABENKIN B. *Microbiological assays for vitamin B_{12} A cyanide enhancement effect* J Biol. Chem. 191 135-141 July 1951.

An improved assay for vitamin B_{12}, utilizing Lactobacillus lactis Dorner ATCC 8000, has been described which incorporates KCN in the basal medium. Results comparable to those obtained with Lactobacillus leichmannii have been obtained on a variety of natural and partially purified vitamin B_{12}-containing materials.

1008. BROWNLEE, K. A., and LAPEDES D N : *The effect of design upon the error of a microbiological assay for vitamin B_{12}* J Bact. 62 433-444, Oct. 1951.

*Squibb Institute for Medical Research
New Brunswick, N. J*

1009. BURKHOLDER, P R.: *Determination of vitamin B_{12} with a mutant strain of Escherichia coli*, Science 114 459-460, Nov 2, 1951

1010. [illegible] ISON E., LEES, K. A., and WOOD, F : *[illegible] y of vitamin B_{12}. Part VI Microbiological [illegible] a with a mutant of Escherichia coli by* [illegible] Analyst 76 696-705, Dec. 1951.

[illegible] *cup assay of vitamin B_{12} [illegible] mutant of Escherichia* [illegible] Lab. Invest. 1: 215-216, [illegible]

1012. TARR, H. L. A.: *Microbiological assay of vitamin B_{12} in crude materials*, Federation Proc. 11 297 298, March 1952.

"Work in two different laboratories has shown that addition of cyanide but no reducing agent to media in titrimetric assays has given similar results with cyano- and hydroxo-cobalamin. Titrimetric procedures are slow and use of cyanide without reducing agent in Skeggs (1950) medium resulted in slow growth, while simultaneous use of reducing agent and cyanide permitted rapid growth but rarely gave the same results with the different cobalamins. Aseptic addition of both cyano- and hydroxo-cobalamin to the medium using 0.5 mg/ml. of mercapto-succinic acid gave identical turbidimetric assay curves, while use of ascorbic acid under such conditions had a pronounced destructive action on hydroxo-cobalamin. Sterilization in the medium normally enhanced growth with cyano-cobalamin but had a marked destructive effect on hydroxo-cobalamin. These findings are important because in crude protein materials much of the vitamin B_{12} occurs as hydroxocobalamin or similar form chromatographically (Woodruff and Foster KH_2PO_4-paper method) When 500 mμg. of cyano- or hydroxo-cobalamin was added to 1-gm. samples of fish meal and these were autoclaved 5 minutes at 120 C., 95-110% of the added vitamin could be recovered by aseptic addition turbidimetric assay Chromatographic separation of cyano- and hydroxo-cobalamins from such preparations (2-5 mμg.) followed by elution and combined assay gave 86-89% recovery With this method of assay heating crude materials with a reducing agent such as mercaptosuccinic acid may cause fictitiously high recovery due to formation of a more reactive form of the vitamin. Proteolysis with enzymes often occasions formation of high concentrations of uncharacterized vitamin B_{12}-active substances."

Pacific Fisheries Experimental Station
Vancouver B. C., Canada

1013 WILLIAMS W L., ESPOSITO R. G., PIERCE, J V *A rapid simp assay method for vitamin B_{12} and other* Federation Proc. 11 458, March 1952.

"The vitamin B_{12}-requiring *E. coli* mutant, 113-3 (Davis and Mingioli *J Bact.* 60: 17 1950 [1034]) has been utilized for the assay of vitamin B_{12} means of a new rapid simplified technique. Test sc are pipetted on filter paper discs and the discs are on the basal medium solidified with agar Zones growth are measured at any time from 12 hours to 3 The basal medium is simple and chemically defined sisting of inorganic salts and glucose. Pads known amounts of vitamin B_{12} can be prepared in vance so that standard curves can be prepared in a fe minutes. Aseptic conditions are not essential. One or less time is required to carry out the pad-plate pr cedure compared to the test-tube technique. The has the fundamental advantage that vitamin B_{12} vitamin B_{12b} as well as amino-cobalamin, nitroso-c lamin and sulfite-treated cyano-cobalamin all have activity Relatively large amounts of methionine will growth of the organism in the absence of vitamin This interference can be readily detected in the pad method by the faint diffuse growth zones exhibited methionine. The pad-plate has also been used fully for the assay of protogen with *Streptococcus* by reversal of propionate inhibition, for the assay of with Lactobacillus bulgaricus, and for the assay of citr vorum factor with *Leuconostoc citrovorum*. Preli experiments with the yeast, *Saccharomyces car* indicate that vitamin B_6, pantothenic acid, inositol biotin can be assayed with this organism using the plate procedure."

Lederle Laboratories
Pearl River N Y

1014 FORD J E.: *The microbiological assay of min B_{12}*, Brit. J Nutrition 6: 324-330, (No. 3) 1952.

BACTERIAL GROWTH, NUTRITION METABOLISM

1015. SHIVE, W., RAVEL, J M., and EAKIN R. E.: *An interrelationship of thymidine and vitamin B_{12}*. J Am. Chem. Soc. 70 2614-2615, July 1948 (Letter to Editor)

A medium containing enzymatic digest of casein is described which supports good growth of Lactobacillus lactis Dorner in the presence of liver extracts containing anti-pernicious anemia principles and can be used as an assay medium. With this medium, or with one containing clarified tomato juice in place of the enzymatic digest of casein, thymidine adequately replaced the liver extracts containing anti-pernicious anemia principles. Half-maximum stimulation of growth was obtained at a concentration of 1 to 3 mcg. thymidine per 10 cc. It therefore appears probable that vitamin B_{12} functions in the biosynthesis of thymidine. When the medium containing tomato juice was utilized, the liver extract was replaced by as little as 1 cc. of sterile, aerated distilled water added aseptically to 10 cc. of medium, and this effect was enhanced by aseptic addition of ascorbic acid. When enzymatic digest of casein replaced the tomato juice, the aerated water was inactive, but 1 mg. of ascorbic in 1 cc. of sterile, aerated water per 10 cc. of medium adequately replaced the liver extracts.

1016. WRIGHT L. D., SKEGGS H. R., and HU J W: *The ability of thymidine to replace min B_{12} as a growth factor for certa.* J Biol. Chem. 175 475-476, Aug. 1948 (Letter t Editor)

Chemical investigation (*J Am. Chem. Soc.* 70 1948) indicates that the crystalline compound functionall related to folic acid and isolated from liver is (*thymine desoxyriboside*) For certain lactic acid bac teria, thymidine is able to replace the requirement min B_{12}. Studies on Lactobacillus lactis are which show that growth comparable to that obtained liver is possible in the presence of 0.4 to 2.0 mcg. midine per tube. Thymine is not active under conditions. The large amounts of thymidine

for optimal growth indicate that thymidine is not vitamin B_{12}. These results are interpreted as indicating that vitamin B_{12} functions as a coenzyme in carrying out reactions concerned with conversion of thymine to thymidine, since in the presence of thymidine the vitamin is no longer required by the organism. This microbiological evidence also indicates that the biochemical defect in pernicious anemia may be the inability to synthesize certain nucleosides, particularly thymidine, from parent purines or pyrimidines, and that the curative effect of folic acid on the disease may be due to increased thymine synthesis, which by mass action effects yields more thymidine. This is further borne out by the effectiveness of large amounts of thymine in pernicious anemia which has been reported by other workers (Spies *et al.*, *South. M J* 39 269 1946)

1017 SHIVE, W., RAVEL, J M., and HARDING W M. *An interrelationship of purines and vitamin B_{12}*, J Biol. Chem. 176 991-992, Nov 1948 (Letter to Editor)

Purines, or derivatives thereof, and thymidine are essential for the growth of Lactobacillus lactis Dorner in the absence of vitamin B_{12}. The purine requirement is less specific than the requirement of thymidine, which cannot be replaced by thymine. Guanylic acid was the most effective of single purines or derivatives, and mixtures of adenine and guanine or of hypoxanthine and guanine also were active. Three different concentrates of *vitamin B_{12} prepared by different processes* replaced both purines and thymidine in the nutrition of the organism. These results indicate that vitamin B_{12} is involved in the biosynthesis of purines (or their derivatives) as well as thymidine. These substances may in turn, be involved in the biosynthesis of vitamin B_{12}.

Experiments on Lactobacillus leichmannii have shown that thymidine can replace the animal protein factor in the nutrition of the organism. This organism also requires folic acid for growth in a medium containing purines. The requirement for folic acid is replaced by thymine after a lag phase. Thymidine in the presence of folic acid replaces the animal protein factor and also slowly replaces both folic acid and the animal protein factor after a lag phase. Thus, independent functions are indicated for folic acid and for the animal protein factor which presumably is identical functionally with vitamin B_{12} in the biosynthesis of thymine and thymidine.

1018. KITAY E., McNUTT W S., and SNELL, E. E. *The non-specificity of thymidine as a growth factor for lactic acid bacteria*, J Biol. Chem. 177 993-994, Feb. 1949 (Letter to Editor)

For 11 of 13 microorganisms thymidine, hypoxanthine desoxyriboside, adenine desoxyriboside, or cytosine desoxyriboside were found to be equally active in promoting growth. Refined liver extract and desoxyribonucleic acid (DNA) were also active for many organisms. Ascorbic acid was active only rarely and at much higher concentrations. Lactobacillus delbrueckii 730 is unique among the organisms tested because it has a specific requirement for thymidine which can not be adequately replaced by the other desoxyribonucleosides or by low levels of liver extract. If the desoxyribonucleosides function by permitting synthesis of DNA, then in most of these organisms this is permitted by either the active factor of liver extract (presumably vitamin B_{12}) or by any one of four desoxyribonucleosides. It is possible that the desoxyribonucleosides function interchangeably by supplying their common portion, either as desoxyribose or as desoxyribose phosphate, for synthesis of DNA, and that this common portion may be formed by independent processes involving vitamin B_{12}.

University of Wisconsin
Madison, Wis.

1019 HOFF JØRGENSEN E.: *Difference in growth promoting effect of desoxyribosides and vitamin B_{12} on three strains of lactic acid bacteria*, J Biol. Chem. 178 525-526, March 1949 (Letter to Editor)

In the course of a survey of the nutritional requirements of lactic acid bacteria of the genus Thermobacterium, a strain, Tbm. acidophilus R26, was found which required thymidine. Neither a B_{12} concentrate nor a commercial liver preparation could replace thymidine for this strain, but for Tbm. lactis 1, charcoal-treated tryptic casein digest was capable of replacing either thymidine or vitamin B_{12}. A third strain, Tbm. juhurt 18, grew on a medium containing either thymidine or vitamin B_{12}, but tryptic casein digest was ineffective. Guanine desoxyriboside had the same effect as thymidine on all three strains.

1020. TOMARELLI, R. M., NORRIS R. F., and GYÖRGY P *Inability of vitamin B_{12} to replace the desoxyriboside requirement of a* Lactobacillus bifidus, J Biol. Chem. 179 485-486, May 1949 (Letter to Editor)

Contrary to the findings with other strains of lactobacilli, vitamin B_{12} even at high levels could not replace the desoxyribosides for the growth of a strain of L. bifidus isolated from the stool of a breast fed infant. Ribonucleic acid, thymine, and ascorbic acid, either alone or in combination with vitamin B_{12}, also were inactive. Growth was obtained on a chemically defined medium supplemented with an enzymatic digest of casein. It is suggested that the crude enzyme preparation contains a factor or factors much more active than the nucleosides. This is not crystalline vitamin B_{12} but may be one of the other six entities demonstrated by Winsten and Eigen (Abstr 1061) in samples possessing vitamin B_{12} or animal protein factor activity

1021 WELCH, A. D., and WILSON M. F *Mechanism of the growth-promoting effect of ascorbic acid on Lactobacillus leichmannii and the reduction of oxidation products of vitamin B$_{12}$*, Arch. Biochem. 22 486-489 July 1949

A digest of vitamin-free casein made with trypsin promotes vigorous growth of Lactobacillus leichmannii in the absence of added vitamin B_{12} and the activity is increased by ascorbic acid treatment. The activity is only slightly altered by heating and is reduced one-half by autoclaving at pH 10. The remaining activity is increased by ascorbic acid treatment. Vitamin B_{12} added prior to autoclaving is not completely inactivated and the residual activity is increased by ascorbic acid. Autoclaving vita-

min B_{12} in water at pH 10 reduces its microbiological activity by over 99 per cent. The apparent microbiological activity of ascorbic acid may be attributed to the occurrence of oxidation products of vitamin B_{12} in impure trypsin and in tryptically digested vitamin-free casein.

Western Reserve University School of Medicine
Cleveland, Ohio

1022. WRIGHT M H *Thymidine and vitamin B_{12}*. Science 110 257 258, Sept. 9 1949

Although thymidine and vitamin B_{12} proved capable of replacing each other in supporting the growth of Lactobacillus leichmannii, data obtained with Leuconostoc citrovorum and Streptococcus fecalis R indicated no specific thymidine–vitamin B-12 relationship.

National Institutes of Health
Bethesda, Md.

1023. FRANKLIN A. L., STOKSTAD E. L. R., HOFFMANN, C. E., BELT M., and JUKES T. H.: *Inhibition of growth of Escherichia coli by 4-aminopteroylglutamic acid and its reversal*, J Am. Chem. Soc. 71 3549-3550, Oct. 1949 (in Notes)

The growth of Escherichia coli was inhibited by high levels of 4-aminopteroylglutamic acid (4-amino PGA) in spite of the fact that the organism does not need pteroyl glutamic acid (PGA) for growth. The inhibition was found to be reversed by liver extract or thymidine but not by PGA, p-aminobenzoic acid, vitamin B_{12}, thymine, guanine, hypoxanthine, adenosine, adenylic acid, cytidylic acid or the desoxyribosides of guanidine and hypoxanthine.

It was also found that Lactobacillus leichmannii 313, which needs both PGA (or p-aminobenzoic acid) and vitamin B_{12} for growth, was inhibited by 4-amino PGA. This inhibition was reversible by PGA at low levels of 4-amino PGA, but at high levels of the latter PGA was ineffective while thymidine produced a reversal. The desoxyribosides of guanine and hypoxanthine were ineffective as reversing agents although they produced approximately maximum growth in the presence of PGA if 4-amino PGA and B_{12} were omitted. In the absence of 4-amino PGA, thymidine produced an incomplete growth response, about 50 per cent of maximum, if PGA and p-aminobenzoic acid were omitted, with or without the addition of vitamin B_{12}.

Lederle Laboratories
Pearl River N Y

1024 ROBERTS, I. Z., ROBERTS R. B., and ABELSON P H. *Effect of vitamin B_{12} on the phosphorus metabolism of Lactobacillus leichmannii*, J Bact. 58 709-710, Nov 1949.

Lactobacillus leichmannii organisms growing in media enriched with vitamin B_{12} absorbed more radioactive phosphorus than those growing in control media. This effect was particularly noticeable in the desoxyribonucleic acid fraction, in which the radioactivity of the vitamin B_{12} sample was about four times that of the control. The authors state that these observations are in accord with the concept that vitamin B_{12} is involved in nucleic acid synthesis.

Carnegie Institution of Washington
Washington, D. C.

1025. SAUBERLICH, H. E.: *The relationship of foli acid vitamin B_{12} and thymidine in the nutritio of Leuconostoc citrovorum 8081* Arch. Biochen 24: 224-232, Nov 1949

In the presence of citrovorum factor concentrates c high concentrations of thymidine, Leuconostoc citro orum 8081 did not require folic acid. However in th presence of folic acid antagonists growth was inhibitec The growth inhibition of 4-amino PGA was reversed b citrovorum factor concentrates, prepared from liver e tracts and rat urine. The concentrates were free of thy midine and low in folic acid. High concentrations of foli acid were weakly effective, vitamin B_{12} was ineffective and thymidine in high concentrations was somewha effective against 4-amino PGA toxicity

Alabama Polytechnic Institute
Auburn, Ala.

1026. SAUBERLICH, H. E., and BAUMANN C. A. *Further studies on the factor required by Leuconostoc citrovorum 8081* J Biol. Chem. 181 871 877 Dec. 1949

Vitamin B_{12} did not stimulate the growth of L citrovorum 8081

1027 KOCHER, V., and SCHINDLER, O *Desoxyribonucleoside als Wachstumsfaktoren fur Lactobacillus lactis in B_{12}-freier Nährlosung (Desoxyribonucleosides as growth factors for Lactobacill lactis in a B_{12}-free medium)* Intern. Z. Vitamin forsch. 20 441-443 1949

Besides thymidine, guanine- cytosine- and hypoxan thine-desoxyribonucleosides may replace B_{12} in the gro of L. lactis.

1028. JUKES T H., BROQUIST H. P., and STOKSTAD E. L. R. *Vitamin B_{12} and "citrovorum factor" in the nutrition of Lactobacillus leichmannii and Leuconostoc citrovorum*, Arch. Biochem. 26 157 159 March 1950.

It has been observed earlier that vitamin B_{12} or the desoxyribosides of guanine, hypoxanthine, adenine, cytosine, or thymine, will promote growth in Lactobacillus leichmannii 313 while "citrovorum factor" (CF) or thymidine, but not the other desoxyribosides or B_{12}, will permit Leuconostoc citrovorum 8081 to grow on a purified culture medium. The authors found that thymidine can replace the requirement of L. leichmannii for both vitamin B_{12} and folic acid. This may indicate that the organism is able to synthesize the other desoxyribosides from thymidine which obviates the need of the organism for vitamin B_{12} in the production of the other desoxyribosides when thymidine is supplied. Vitamin B_{12} formed by Leuconostoc citrovorum may serve in the synthesis of desoxyribosides other than thymidine which in turn act as precursors of thymidine when CF is supplied. With L. leichmannii, CF produced by the organism may catalyze the formation of thymidine from the other desoxyribosides which are formed when B_{12} is supplied.

1029 OGINSKY E. L. *Vitamin B_{12} and methionine formation*, Arch. Biochem. 26 327-329 April 1950.

for optimal growth indicate that thymidine is not vitamin B_{12}. These results are interpreted as indicating that vitamin B_{12} functions as a coenzyme in carrying out reactions concerned with conversion of thymine to thymidine, since in the presence of thymidine the vitamin is no longer required by the organism. This microbiological evidence also indicates that the biochemical defect in pernicious anemia may be the inability to synthesize certain nucleosides, particularly thymidine, from parent purines or pyrimidines, and that the curative effect of folic acid on the disease may be due to increased thymine synthesis, which by mass action effects yields more thymidine. This is further borne out by the effectiveness of large amounts of thymine in pernicious anemia which has been reported by other workers (Spies *et al.*, *South. M J* 39 269 1946)

1017 SHIVE, W., RAVEL, J M., and HARDING, W M.: *An interrelationship of purines and vitamin B_{12}*, J Biol. Chem. 176 991-992, Nov 1948 (Letter to Editor)

Purines, or derivatives thereof, and thymidine are essential for the growth of Lactobacillus lactis Dorner in the absence of vitamin B_{12}. The purine requirement is less specific than the requirement of thymidine, which cannot be replaced by thymine. Guanylic acid was the most effective of single purines or derivatives, and mixtures of adenine and guanine or of hypoxanthine and guanine also were active. Three different concentrates of vitamin B_{12} prepared by different processes replaced both purines and thymidine in the nutrition of the organism. These results indicate that vitamin B_{12} is involved in the biosynthesis of purines (or their derivatives) as well as thymidine. These substances may in turn, be involved in the biosynthesis of vitamin B_{12}.

Experiments on Lactobacillus leichmannii have shown that thymidine can replace the animal protein factor in the nutrition of the organism. This organism also requires folic acid for growth in a medium containing purines. The requirement for folic acid is replaced by thymine after a lag phase. Thymidine in the presence of folic acid replaces the animal protein factor and also slowly replaces both folic acid and the animal protein factor after a lag phase. Thus, independent functions are indicated for folic acid and for the animal protein factor which presumably is identical functionally with vitamin B_{12} in the biosynthesis of thymine and thymidine.

1018. KITAY E., McNUTT W S., and SNELL, E. E.: *The non specificity of thymidine as a growth factor for lactic acid bacteria*, J Biol. Chem. 177 993-994, Feb. 1949 (Letter to Editor)

For 11 of 13 microorganisms thymidine, hypoxanthine desoxyriboside, adenine desoxyriboside, or cytosine desoxyriboside were found to be equally active in promoting growth. Refined liver extract and desoxyribonucleic acid (DNA) were also active for many organisms. Ascorbic acid was active only rarely and at much higher concentrations. Lactobacillus delbrueckii 730 is unique among the organisms tested because it has a specific requirement for thymidine which can not be adequately replaced by the other desoxyribonucleosides or by low levels of liver extract. If the desoxyribonucleosides function by permitting synthesis of DNA, then in most of these organisms this is permitted by either the active factor of liver extract (presumably vitamin B_{12}) or by any one of four desoxyribonucleosides. It is possible that the desoxyribonucleosides function interchangeably by supplying their common portion, either as desoxyribose or as desoxyribose phosphate, for synthesis of DNA, and that this common portion may be formed by independent processes involving vitamin B_{12}.

University of Wisconsin
Madison, Wis.

1019 HOFF JØRGENSEN E.: *Difference in growth-promoting effect of desoxyribosides and vitamin B_{12} on three strains of lactic acid bacteria*, J Biol. Chem. 178 525-526, March 1949 (Letter to Editor)

In the course of a survey of the nutritional requirements of lactic acid bacteria of the genus Thermobacterium, a strain, Thm. acidophilus R26, was found which required thymidine. Neither a B_{12} concentrate nor a commercial liver preparation could replace thymidine for this strain, but for Thm. lactis 1, charcoal-treated tryptic casein digest was capable of replacing either thymidine or vitamin B_{12}. A third strain, Thm. Jahurt 13, grew on a medium containing either thymidine or vitamin B_{12}, but tryptic casein digest was ineffective. Guanine desoxyriboside had the same effect as thymidine on all three strains.

1020. TOMARELLI, R. M., NORRIS, R. F., and GYÖRGY P: *Inability of vitamin B_{12} to replace the desoxyriboside requirement of a Lactobacillus bifidus*, J Biol. Chem. 179 485-486, May 1949 (Letter to Editor)

Contrary to the findings with other strains of lactobacilli, vitamin B_{12} even at high levels could not replace the desoxyribosides for the growth of a strain of L. bifidus isolated from the stool of a breast fed infant. Ribonucleic acid, thymine, and ascorbic acid, either alone or in combination with vitamin B_{12}, also were inactive. Growth was obtained on a chemically defined medium supplemented with an enzymatic digest of casein. It is suggested that the crude enzyme preparation contains a factor or factors much more active than the nucleosides. This is not crystalline vitamin B_{12} but may be one of the other six entities demonstrated by Winsten and Eigen (Abstr 1061) in samples possessing vitamin B_{12} or animal protein factor activity

1021 WELCH, A. D., and WILSON M. F: *Mechanism of the growth-promoting effect of ascorbic acid on Lactobacillus leichmannii and the reduction of oxidation products of vitamin B_{12}*, Arch. Biochem. 22 486-489 July 1949

A digest of vitamin-free casein made with trypsin promotes vigorous growth of Lactobacillus leichmannii in the absence of added vitamin B_{12} and the activity is increased by ascorbic acid treatment. The activity is only slightly altered by heating and is reduced one-half by autoclaving at pH 10. The remaining activity is increased by ascorbic acid treatment. Vitamin B_{12} added prior to autoclaving is not completely inactivated and the residual activity is increased by ascorbic acid. Autoclaving vita-

min B_{12} in water at pH 10 reduces its microbiological activity by over 99 per cent. The apparent microbiological activity of ascorbic acid may be attributed to the occurrence of oxidation products of vitamin B_{12} in impure trypsin and in tryptically digested vitamin-free casein.

Western Reserve University School of Medicine
Cleveland, Ohio

1022. WRIGHT M. H. *Thymidine and vitamin B_{12}.* Science 110 257 258, Sept. 9 1949

Although thymidine and vitamin B_{12} proved capable of replacing each other in supporting the growth of Lactobacillus leichmannii, data obtained with Leuconostoc citrovorum and Streptococcus fecalis R indicated no specific thymidine–vitamin B-12 relationship.

National Institutes of Health
Bethesda, Md.

1023 FRANKLIN A. L., STOKSTAD E. L. R., HOFFMANN C. E., BELT M., and JUKES, T. H. *Inhibition of growth of Escherichia coli by 4-aminopteroylglutamic acid and its reversal,* J Am. Chem. Soc. 71 3549-3550 Oct. 1949 (In Notes)

The growth of Escherichia coli was inhibited by high levels of 4-aminopteroylglutamic acid (4-amino PGA) in spite of the fact that the organism does not need pteroylglutamic acid (PGA) for growth. The inhibition was found to be reversed by liver extract or thymidine but not by PGA, p-aminobenzoic acid, vitamin B_{12}, thymine, guanine, hypoxanthine, adenosine, adenylic acid, cytidylic acid or the desoxyribosides of guanidine and hypoxanthine.

It was also found that Lactobacillus leichmannii 313, which needs both PGA (or p-aminobenzoic acid) and vitamin B_{12} for growth, was inhibited by 4-amino PGA. This inhibition was reversible by PGA at low levels of 4-amino PGA, but at high levels of the latter PGA was ineffective while thymidine produced a reversal. The desoxyribosides of guanine and hypoxanthine were ineffective as reversing agents although they produced approximately maximum growth in the presence of PGA if 4-amino PGA and B_{12} were omitted. In the absence of 4-amino PGA, thymidine produced an incomplete growth response, about 50 per cent of maximum, if PGA and p-aminobenzoic acid were omitted, with or without the addition of vitamin B_{12}.

Lederle Laboratories
Pearl River N Y

1024 ROBERTS, L Z., ROBERTS R. B., and ABELSON P H. *Effect of vitamin B_{12} on the phosphorus metabolism of Lactobacillus leichmannii,* J Bact. 58 709-710, Nov 1949

Lactobacillus leichmannii organisms growing in media enriched with vitamin B_{12} absorbed more radioactive phosphorus than those growing in control media. This effect was particularly noticeable in the desoxyribonucleic acid fraction, in which the radioactivity of the vitamin B_{12} sample was about four times that of the control. The authors state that these observations are in accord with the concept that vitamin B_{12} is involved in nucleic acid synthesis.

Carnegie Institution of Washington
Washington, D. C.

1025 SAUBERLICH, H. E. *The relationship of folic acid vitamin B_{12} and thymidine in the nutrition of Leuconostoc citrovorum 8081* Arch. Biochem. 24 224-232, Nov 1949

In the presence of citrovorum factor concentrates or high concentrations of thymidine, Leuconostoc citrovorum 8081 did not require folic acid. However in the presence of folic acid antagonists growth was inhibited. The growth inhibition of 4-amino PGA was reversed by citrovorum factor concentrates, prepared from liver extracts and rat urine. The concentrates were free of thymidine and low in folic acid. High concentrations of folic acid were weakly effective, vitamin B_{12} was ineffective, and thymidine in high concentrations was somewhat effective against 4-amino PGA toxicity

Alabama Polytechnic Institute
Auburn, Ala.

1026. SAUBERLICH, H. E., and BAUMANN C. A. *Further studies on the factor required by Leuconostoc citrovorum 8081* J Biol. Chem. 181 871 877 Dec. 1949

Vitamin B_{12} did not stimulate the growth of L. citrovorum 8081

1027 KOCHER, V., and SCHINDLER, O *Desoxyribonucleoside als Wachstumsfaktoren fur Lactobacillus lactis in B_{12}-freier Nahrlosung (Desoxyribonucleosides as growth factors for Lactobacillus lactis in a B_{12}-free medium)* Intern. Z. Vitaminforsch. 20 441-443 1949

Besides thymidine, guanine- cytosine- and hypoxanthine-desoxyribonucleosides may replace B_{12} in the growth of L. lactis.

1028. JUKES T H., BROQUIST H. P., and STOKSTAD E. L. R. *Vitamin B_{12} and "citrovorum factor" in the nutrition of Lactobacillus leichmannii and Leuconostoc citrovorum,* Arch. Biochem. 26 157 159 March 1950.

It has been observed earlier that vitamin B_{12} or the desoxyribosides of guanine, hypoxanthine, adenine, cytosine, or thymine, will promote growth in Lactobacillus leichmannii 313 while "citrovorum factor" (CF) or thymidine, but not the other desoxyribosides or B_{12}, will permit Leuconostoc citrovorum 8081 to grow on a purified culture medium. The authors found that thymidine can replace the requirement of L. leichmannii for both vitamin B_{12} and folic acid. This may indicate that the organism is able to synthesize the other desoxyribosides from thymidine which obviates the need of the organism for vitamin B_{12} in the production of the other desoxyribosides when thymidine is supplied. Vitamin B_{12} formed by Leuconostoc citrovorum may serve in the synthesis of desoxyribosides other than thymidine which in turn act as precursors of thymidine when CF is supplied. With L. leichmannii, CF produced by the organism may catalyze the formation of thymidine from the other desoxyribosides which are formed when B_{12} is supplied.

1029 OGINSKY E. L. *Vitamin B_{12} and methionine formation,* Arch. Biochem. 26 327-329 April 1950.

for optimal growth indicate that thymidine is not vitamin B_{12}. These results are interpreted as indicating that vitamin B_{12} functions as a coenzyme in carrying out reactions concerned with conversion of thymine to thymidine, since in the presence of thymidine the vitamin is no longer required by the organism. This microbiological evidence also indicates that the biochemical defect in pernicious anemia may be the inability to synthesize certain nucleosides, particularly thymidine, from parent purines or pyrimidines, and that the curative effect of folic acid on the disease may be due to increased thymine synthesis, which by mass action effects yields more thymidine. This is further borne out by the effectiveness of large amounts *of thymine in pernicious anemia which has been reported* by other workers (Spies *et al., South. M J* 39 269 1946)

1017 SHIVE, W., RAVEL, J M., and HARDING, W M *An interrelationship of purines and vitamin B_{12}*, J Biol. Chem. 176 991 992, Nov 1948 (Letter to Editor)

Purines, or derivatives thereof, and thymidine are essential for the growth of Lactobacillus lactis Dorner in the absence of vitamin B_{12}. The purine requirement is less specific than the requirement of thymidine, which cannot be replaced by thymine. Guanylic acid was the most effective of single purines or derivatives, and mixtures of adenine and guanine or of hypoxanthine and guanine also were active. *Three different concentrates of* vitamin B_{12} prepared by different processes replaced both purines and thymidine in the nutrition of the organism. These results indicate that vitamin B_{12} is involved in the biosynthesis of purines (or their derivatives) as well as thymidine. These substances may in turn, be involved in the biosynthesis of vitamin B_{12}.

Experiments on Lactobacillus leichmannii have shown that thymidine can replace the animal protein factor in the nutrition of the organism. This organism also requires folic acid for growth in a medium containing purines. The requirement for folic acid is replaced by thymine after a lag phase. Thymidine in the presence of folic acid replaces the animal protein factor and also slowly replaces both folic acid and the animal protein factor after a lag phase. Thus, independent functions are indicated for folic acid and for the animal protein factor which presumably is identical functionally with vitamin B_{12} in the biosynthesis of thymine and thymidine.

1018. KITAY E., McNUTT W S., and SNELL, E. E.: *The non-specificity of thymidine as a growth factor for lactic acid bacteria*, J Biol. Chem. 177 993-994 Feb. 1949 (Letter to Editor)

For 11 of 13 microorganisms thymidine, hypoxanthine desoxyriboside, adenine desoxyriboside, or cytosine desoxyriboside were found to be equally active in promoting growth. Refined liver extract and desoxyribonucleic acid (DNA) were also active for many organisms. Ascorbic acid was active only rarely and at much higher concentrations. Lactobacillus delbrueckii 730 is unique among the organisms tested because it has a specific requirement for thymidine which can not be adequately replaced by the other desoxyribonucleosides or by low levels of liver extract. If the desoxyribonucleosides function by permitting synthesis of DNA, then in most of these organisms this is permitted by either the active factor of liver extract (presumably vitamin B_{12}) or by any one of four desoxyribonucleosides. It is possible that the desoxyribonucleosides function interchangeably by supplying their common portion, either as desoxyribose or as desoxyribose phosphate, for synthesis of DNA, and that this common portion may be formed by independent processes involving vitamin B_{12}.

University of Wisconsin
Madison, Wis.

1019 HOFF JØRGENSEN E.: *Difference in growth promoting effect of desoxyribosides and vitamin B_{12} on three strains of lactic acid bacteria*, J Biol. Chem. 178 525-526, March 1949 (Letter to Editor)

In the course of a survey of the nutritional requirements of lactic acid bacteria of the genus Thermobacterium, a strain, Thm. acidophilus R26, was found which required thymidine. Neither a B_{12} concentrate nor a commercial liver preparation could replace thymidine for this strain, but for Thm. lactis 1, charcoal-treated tryptic *casein digest was capable of replacing either thymidine* or vitamin B_{12}. A third strain, Thm. Juhurt 13, grew on a medium containing either thymidine or vitamin B_{12}, but tryptic casein digest was ineffective. Guanine desoxyriboside had the same effect as thymidine on all three strains.

1020. TOMARELLI, R. M., NORRIS, R. F., and GYÖRGY P *Inability of vitamin B_{12} to replace the desoxyriboside requirement of a* Lactobacillus bifidus, J Biol. Chem. 179 485-486, May 1949 (Letter to Editor)

Contrary to the findings with other strains of lactobacilli, vitamin B_{12} even at high levels could not replace the desoxyribosides for the growth of a strain of L. bifidus isolated from the stool of a breast fed infant. Ribonucleic acid, thymine, and ascorbic acid, either alone or in combination with vitamin B_{12}, also were inactive. Growth was obtained on a chemically defined medium supplemented with an enzymatic digest of casein. It is suggested that the crude enzyme preparation contains a factor or factors much more active than the nucleosides. This is not crystalline vitamin B_{12} but may be one of the other six entities demonstrated by Winsten and Eigen (Abstr 1061) in samples possessing vitamin B_{12} or animal protein factor activity

1021. WELCH, A. D., and WILSON M. F *Mechanism of the growth-promoting effect of ascorbic acid on Lactobacillus leichmannii and the reduction of oxidation products of vitamin B_{12}*, Arch. Biochem. 22 486-489 July 1949

A digest of vitamin-free casein made with *trypsin* promotes vigorous growth of Lactobacillus leichmannii in the absence of added vitamin B_{12} and the activity is increased by ascorbic acid treatment. The activity is only slightly altered by heating and is reduced one-half by autoclaving at pH 10. The remaining activity is increased by ascorbic acid treatment. Vitamin B_{12} added prior to autoclaving is not completely inactivated and the residual activity is increased by ascorbic acid. Autoclaving vita-

min B_{12} in water at pH 10 reduces its microbiological activity by over 99 per cent. The apparent microbiological activity of ascorbic acid may be attributed to the occurrence of oxidation products of vitamin B_{12} in impure trypsin and in tryptically digested vitamin free casein.

Western Reserve University School of Medicine
Cleveland, Ohio

1022. WRIGHT M. H *Thymidine and vitamin B_{12}*, Science 110: 257 258, Sept. 9 1949

Although thymidine and vitamin B_{12} proved capable of replacing each other in supporting the growth of Lactobacillus leichmannii, data obtained with Leuconostoc citrovorum and Streptococcus fecalis R indicated no specific thymidine–vitamin B-12 relationship.

National Institutes of Health
Bethesda, Md.

1023. FRANKLIN A. L., STOKSTAD E. L. R., HOFFMANN C. E., BELT M., and JUKES T. H. *Inhibition of growth of Escherichia coli by 4-aminopteroylglutamic acid and its reversal*, J Am. Chem. Soc. 71 3549-3550, Oct. 1949 (In Notes)

The growth of Escherichia coli was inhibited by high levels of 4-aminopteroylglutamic acid (4-amino PGA) in spite of the fact that the organism does not need pteroylglutamic acid (PGA) for growth. The inhibition was found to be reversed by liver extract or thymidine but not by PGA, p-aminobenzoic acid, vitamin B_{12}, thymine, guanine, hypoxanthine, adenosine, adenylic acid, cytidylic acid or the desoxyribosides of guanidine and hypoxanthine.

It was also found that Lactobacillus leichmannii 313, which needs both PGA (or p-aminobenzoic acid) and vitamin B_{12} for growth, was inhibited by 4-amino PGA. This inhibition was reversible by PGA at low levels of 4-amino PGA, but at high levels of the latter PGA was ineffective while thymidine produced a reversal. The desoxyribosides of guanine and hypoxanthine were ineffective as reversing agents although they produced approximately maximum growth in the presence of PGA if 4-amino PGA and B_{12} were omitted. In the absence of 4-amino PGA, thymidine produced an incomplete growth response, about 50 per cent of maximum, if PGA and p-aminobenzoic acid were omitted, with or without the addition of vitamin B_{12}.

Lederle Laboratories
Pearl River N. Y.

1024 ROBERTS, L. Z., ROBERTS R. B., and ABELSON P. H. *Effect of vitamin B_{12} on the phosphorus metabolism of Lactobacillus leichmannii*, J Bact. 58 709-710 Nov 1949

Lactobacillus leichmannii organisms growing in media enriched with vitamin B_{12} absorbed more radioactive phosphorus than those growing in control media. This effect was particularly noticeable in the desoxyribonucleic acid fraction, in which the radioactivity of the vitamin B_{12} sample was about four times that of the control. The authors state that these observations are in accord with the concept that vitamin B_{12} is involved in nucleic acid synthesis.

Carnegie Institution of Washington
Washington, D. C.

1025 SAUBERLICH, H. E. *The relationship of folic acid vitamin B_{12} and thymidine in the nutrition of Leuconostoc citrovorum 8081* Arch. Biochem. 24 221-232, Nov 1949

In the presence of citrovorum factor concentrates or high concentrations of thymidine, Leuconostoc citrovorum 8081 did not require folic acid. However in the presence of folic acid antagonists growth was inhibited. The growth inhibition of 4-amino PGA was reversed by citrovorum factor concentrates, prepared from liver extracts and rat urine. The concentrates were free of thymidine and low in folic acid. High concentrations of folic acid were weakly effective, vitamin B_{12} was ineffective, and thymidine in high concentrations was somewhat effective against 4-amino PGA toxicity

Alabama Polytechnic Institute
Auburn, Ala.

1026. SAUBERLICH, H. E., and BAUMANN C. A.: *Further studies on the factor required by Leuconostoc citrovorum 8081* J Biol. Chem. 181 871 877 Dec. 1949

Vitamin B_{12} did not stimulate the growth of L. citrovorum 8081.

1027 KOCHER, V., and SCHINDLER, O *Desoxyribonucleoside als Wachstumsfaktoren fur Lactobacillus lactis in B_{12}-freier Nährlosung (Desoxyribonucleosides as growth factors for Lactobacillus lactis in a B_{12}-free medium)* Intern. Z. Vitaminforsch. 20 441-443, 1949

Besides thymidine, guanine- cytosine- and hypoxanthine-desoxyribonucleosides may replace B_{12} in the growth of L. lactis.

1028. JUKES, T H., BROQUIST H. P., and STOKSTAD E. L. R. *Vitamin B_{12} and "citrovorum factor" in the nutrition of Lactobacillus leichmannii and Leuconostoc citrovorum*, Arch. Biochem. 26 157 159 March 1950.

It has been observed earlier that vitamin B_{12} or the desoxyribosides of guanine, hypoxanthine, adenine, cytosine, or thymine, will promote growth in Lactobacillus leichmannii 313 while "citrovorum factor" (CF) or thymidine, but not the other desoxyribosides or B_{12}, will permit Leuconostoc citrovorum 8081 to grow on a purified culture medium. The authors found that thymidine can replace the requirement of L. leichmannii for both vitamin B_{12} and folic acid. This may indicate that the organism is able to synthesize the other desoxyribosides from thymidine which obviates the need of the organism for vitamin B_{12} in the production of the other desoxyribosides when thymidine is supplied. Vitamin B_{12} formed by Leuconostoc citrovorum may serve in the synthesis of desoxyribosides other than thymidine which in turn act as precursors of thymidine when CF is supplied. With L. leichmannii, CF produced by the organism may catalyze the formation of thymidine from the other desoxyribosides which are formed when B_{12} is supplied.

1029 OGINSKY E. L. *Vitamin B_{12} and methionine formation*, Arch. Biochem. 26 327-329 April 1950.

1030. HENDLIN D., and RUGER, M. L. *The effect of cobalt on the microbial synthesis of LLD-active substances*, Science 111: 541-542, May 19 1950.

In an attempt to increase the microbial synthesis of vitamin B_{12}, cobalt—in the form of $CO(NO_3)_2 \cdot 6H_2O$—was used to supplement the culture medium used. In experiments with Streptomyces griseus, it was found that the addition of cobalt ion (Co^{++}) to the basal medium increased the LLD titer about three-fold. An increase in vitamin B_{12} production was also observed which was approximately parallel to the increase in LLD activity. As little as 1 to 2 ppm. of Co^{++} produced maximal LLD activities. At levels of 20 to 50 ppm. of Co^{++} toxic manifestations were noted, with a concomitant decrease in growth of the microorganisms and in LLD activity.

An extensive screening program has shown that large numbers of microorganisms synthesize LLD-active substances. In a study of some of these (including some isolated from cow rumen contents and cow manure) it was found that the addition of Co^{++} (2 ppm.) resulted in a significant increase in the LLD titer of the broth.

Merck & Co., Inc.
Rahway, N. J.

1031. EMERY W. B., LEES, K. A., and WALKER, A. D.: *Observations on a growth factor for Leuconostoc citrovorum*, Biochem. J. 46: 572-574, May 1950.

Concentrates of a growth factor for Leuconostoc citrovorum contained in liver extracts and fermentation liquors for S. griseus have been prepared and purified. Vitamin B_{12} was removed by an ion-exchange process. The factor is not identical with vitamin B_{12} or B_{13}; it has no activity against pernicious anemia, but possesses leukocyte-stimulating activity.

1032. KITAY E., McNUTT W. S., and SNELL, E. E. *Desoxyribosides and vitamin B_{12} as growth factors for lactic acid bacteria*, J. Bact. 59: 727-738, June 1950.

Eighteen strains of lactic acid bacteria, representative of six species, were tested for certain additional nutritional requirements. None of them grew in a medium complete with respect to known amino acids and synthetic vitamins and supplemented with tomato juice and an enzymatic digest of casein. All grew when thymidine was added to this medium. In most cases, thymidine could be replaced by certain other desoxyribosides. Several strains grew better with thymidine than with other desoxyribosides, and one (Leuconostoc citrovorum 8081) grew only with thymidine. Most organisms showed delayed growth with desoxyribonucleic acid.

Vitamin B_{12} replaced thymidine (or other desoxyribosides) for many, but not all of these organisms. L. delbrueckii 730 and Lactobacillus acidophilus 204 are examples of organisms that respond to thymidine but not to vitamin B_{12}.

Ascorbic acid and certain other reducing agents replaced desoxyribosides or vitamin B_{12} for many of these organisms when the media contained enzymatic digest of casein and for some when it did not. Vitamin B_{12} was effective as vitamin B_{12} when it was added aseptically to previously autoclaved media, but only one-seventh as effective when it was added to the medium before sterilization. All organisms required similar amounts of the desoxyribosides for growth, but their requirements for vitamin B_{12} varied.

University of Wisconsin
Madison, Wis.

1033. KITAY E., and SNELL, E. E.: *Some additional nutritional requirements of certain lactic acid bacteria*, J. Bact. 60: 49-56, July 1950.

In experiments on the nutritive requirements of lactic acid bacteria previously reported not to grow in media of known composition, 18 of the 28 cultures required thymidine, other desoxyribosides, or vitamin B_{12} for growth.

University of Wisconsin
Madison, Wis.

1034. DAVIS, B. D., and MINGIOLI, E. S.: *Mutants of Escherichia coli requiring methionine or vitamin B_{12}*, J. Bact. 60: 17-28, July 1950.

A number of mutants of Escherichia coli have been isolated that require vitamin B_{12} or methionine. They do not respond to homocysteine, the immediate precursor of methionine, and mutants which do respond to homocysteine do not respond to B_{12}. Most of the mutants grow equally rapidly on B_{12} or methionine, but two grow rapidly on methionine and slowly on B_{12}. Vitamin B_{12} appears to be concerned with the methylation of homocysteine. The mutants are stable enough to be used for vitamin B_{12} assay. The quantitative response to B_{12} is greater and more prolonged in shaken than in unshaken tubes. With such assays it could be shown that suspensions of wild type Esch. coli rapidly absorb large amounts of vitamin B_{12} from the medium.

Cornell University Medical College
New York, N. Y.

1035. LANG, C. A., and CHOW B. F.: *Inactivation of microbiological activity of crystalline vitamin B_{12} by reducing agents*, Proc. Soc. Exper. Biol. & Med. 75: 39-41, Oct. 1950.

When a solution of crystalline vitamin B_{12} was subjected to a series of reducing agents (cysteine, ascorbic acid, thiamine, hydroquinone) between pH 4 and 7, at which range this vitamin is stable, a marked loss of the microbiologic activity occurred. *This loss can be* attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color. The APF activity of the solution was not reduced to the same extent as the microbiologic activity.

Johns Hopkins University
Baltimore, Md.

1036. ROBBINS, W. J., HERVEY A., and STEBBINS, M. E.: *Studies on Euglena and vitamin B_{12}*, Science 112: 455, Oct. 20, 1950.

The use of Euglena gracilis var. bacillaris in the bioassay of vitamin B_{12} is reported. It was found that many bacteria and actinomycetes in the soil can synthesize vitamin B_{12}. Yeasts and filamentous fungi apparently are less commonly able to do so. When one part

of fresh soil was shaken with two parts of water the extracts contained amounts of vitamin B_{12} similar to those in cow's milk. The roots of many higher plants contain vitamin B_{12} in amounts ranging from 0 0002 to 0.01 mcg /Gm. of fresh material. It is believed that the vitamin B_{12} is absorbed from soil solutions. A few stems have been shown to contain vitamin B_{12} leaves and fruits did not. It was found that pond water on which Euglena develops regularly contained vitamin B_{12} throughout the year in concentrations of 0.0001 to 0.001 mcg/cc. The pond mud was shown to contain bacteria and actinomycetes capable of vitamin B_{12} synthesis. The authors believe that the original source of vitamin B_{12} in nature is the synthesizing activity of bacteria and actinomycetes, and not that of higher plants.

New York Botanical Gardens
New York, N Y

1037 WEINBERG E. D: *Vitamin requirements of dwarf colony variants of bacteria,* J Infect. Dis. 87 299-306, Nov Dec. 1950.

Attempts were made to induce dwarf strains of bacteria to form large colonies. For this purpose certain additions to plain nutrient agar (on which only tiny colonies were produced) were made. Corynebacterium produced large colonies when thiamine was added, Alcaligenes faecalis when thiamine plus vitamin B_{12} was added, Alcaligenes faecalis when reticulogen was added, and Shigella sonnei when yeast extract was added. When again subcultured on plain nutrient agar the cells of large colonies of all the dwarf strains formed only tiny colonies.

University of Chicago
Chicago, Ill.

1038. REGE, D V., and SREENIVASAN A. *Folic acid vitamin B_{12} and nucleic acid synthesis in Lactobacillus casei,* Nature 166 1117 Dec. 30, 1950.

The effects of vitamin B_{12} and folic acid on nucleic acid metabolism were investigated by growing Lactobacillus casei in media containing varying concentrations of these vitamins, singly or together Folic acid was found to enhance the synthesis of desoxyribonucleic acid. This was also true of vitamin B_{12}, although the latter could not completely replace folic acid as a growth factor of L. casei. The most interesting observation, however was that there is a pronounced and more or less all-around additive effect of folic acid and vitamin B_{12}.

University of Bombay
Bombay, India

1039. WOOLLEY D W *Inhibition of synthesis of vitamin B_{12} and of riboflavin by 1,2-dichloro-4,5-diaminobenzene in bacterial cultures,* Proc. Soc. Exper Biol. & Med. 75 745-746, Dec. 1950.

The amounts of vitamin B_{12} and of riboflavin formed in cultures of Bacillus megatherium were decreased by 1,2-dichloro-4,5-diaminobenzene in quantities insufficient to inhibit growth. The production of vitamin B_{12} was stimulated by the dimethyl analog of diaminobenzene. These facts are regarded as compatible with the previously expressed postulates that the dimethyldiamine is a precursor of riboflavin and of vitamin B_{12}, and that the dichloro analog exerts its selective toxicity at least in part, by inhibition of biosynthetical processes in the formation of these vitamins.

Rockefeller Institute for Medical Research
New York, N Y

1040 WOOLLEY D W *Selective toxicity of 1,2-dichloro-4,5-diaminobenzene its relation to requirements for riboflavin and vitamin B_{12}* J Exper Med 93: 13-24 Jan. 1951

Author's summary: "In a series of 26 species [of organisms] selected from widely differing classes, 1,2-dichloro-4,5-diaminobenzene was toxic to those which did not exhibit a nutritional need for riboflavin plus vitamin B_{12}. It failed to retard the growth of those which needed both of these vitamins. The compound was conceived as an antimetabolite of 1,2-dimethyl-4,5-diaminobenzene. This latter which is contained within the structures of the two vitamins, was pictured as a metabolic precursor of them. It was found to have very slight activity as either riboflavin or as vitamin B_{12} for lactic acid bacteria and algae. The growth inhibiting action of the dichlorodiaminobenzene was overcome competitively by the dimethyldiaminobenzene, and also, to a lesser extent, by o-phenylenediamine. The toxicity was not influenced by additions of riboflavin plus vitamin B_{12}, except in the cases of two species, where the influence was slight. These facts were considered to support the idea that properly constructed analogs of a precursor of two or more essential participants in cell division may be able to circumvent the counteraction which the vitamin has been found to exert on an antimetabolite of its precursor Alternate explanations of the observed data were likewise considered."

Rockefeller Institute for Medical Research
New York, N Y

1041. WEYGAND F., WACKER, A., and WIRTH, F *Relations between vitamin B_{12}, purines and pyrimidines in connection with Lactobacillus leichmannii 313* Z. Naturforschung 6b 25-34 Jan. Feb. 1951.

The requirement of L. leichmannii for purines and pyrimidines has been determined. For optimal growth only guanine (I) and uracil are needed. In the absence of I or xanthine, adenine (II) inhibits the growth, stimulated by B_{12} or desoxyribosides. It is concluded that the organism can not transform II into I 4-Amino- and 5-methyl-4-amino-2-thiouracils, benzimidazole and its dimethyl, dibromo and dichloro derivatives also inhibit growth in the absence of II This growth inhibition is competitively antagonized by II and its derivatives and noncompetitively by B_{12}.

1042. SHIVE, W., SIBLEY M. E., and ROGERS L. L. *Replacement of vitamin B_{12} by desoxynucleotides in promoting growth of certain lactobacilli* J Am. Chem. Soc. 73 867-868, Feb. 1951 (in Notes)

The authors state, in part "Although vitamin B_{12} is approximately 10,000 to 80,000 times as effective as the desoxynucleotides in stimulating growth of the lactobacilli in test tubes, the effect of the desoxynucleotides cannot be accounted for on the basis of contamination

1030. HENDLIN D., and RUGER, M. L.: *The effect of cobalt on the microbial synthesis of LLD-active substances* Science 111 541-542, May 19 1950

In an attempt to increase the microbial synthesis of vitamin B_{12}, cobalt—in the form of $CO(NO_3)_2.6H_2O$—was used to supplement the culture medium used. In experiments with Streptomyces griseus, it was found that the addition of cobalt ion (Co++) to the basal medium increased the LLD titer about three-fold. An increase in vitamin B_{12} production was also observed which was approximately parallel to the increase in LLD activity As little as 1 to 2 ppm. of Co++ produced maximal LLD activities. At levels of 20 to 50 ppm. of Co++ toxic manifestations were noted, with a concomitant decrease in growth of the microorganisms and in LLD activity

An extensive screening program has shown that large numbers of microorganisms synthesize LLD-active substances. In a study of some of these (including some isolated from cow rumen contents and cow manure) it was found that the addition of Co++ (2 ppm.) resulted in a significant increase in the LLD titer of the broth.

Merck & Co., Inc.
Rahway N J

1031 EMERY W B., LEES K. A., and WALKER, A. D: *Observations on a growth factor for Leuconostoc citrovorum*, Biochem. J 46 572-574 May 1950.

Concentrates of a growth factor for Leuconostoc citrovorum contained in liver extracts and fermentation liquors for S griseus have been prepared and purified. Vitamin B_{12} was removed by an ion-exchange process. The factor is not identical with vitamin B_{12} or B_{12} it has no activity against pernicious anemia, but possesses leukocyte-stimulating activity

1032. KITAY E., McNUTT W S., and SNELL, E. E.: *Desoxyribosides and vitamin B_{12} as growth factors for lactic acid bacteria*, J Bact. 59 727 738, June 1950.

Eighteen strains of lactic acid bacteria, representative of six species, were tested for certain additional nutritional requirements. None of them grew in a medium complete with respect to known amino acids and synthetic vitamins and supplemented with tomato juice and an enzymatic digest of casein. All grew when thymidine was added to this medium. In most cases, thymidine could be replaced by certain other desoxyribosides. Several strains grew better with thymidine than with other desoxyribosides, and one (Leuconostoc citrovorum 8081) grew only with thymidine. Most organisms showed delayed growth with desoxyribonucleic acid.

Vitamin B_{12} replaced thymidine (or other desoxyribosides) for many but not all of these organisms. L. delbrueckii 730 and Lactobacillus acidophilus 204 are examples of organisms that respond to thymidine but not to vitamin B_{12}.

Ascorbic acid and certain other reducing agents replaced desoxyribosides or vitamin B_{12} for many of these organisms when the media contained enzymatic digest of casein and for some when it did not. Vitamin B -, was effective as vitamin B_{12} when it was added aseptically to previously autoclaved media, but only one-seventh as effective when it was added to the medium before sterilization. All organisms required similar amounts of the desoxyribosides for growth, but their requirements for vitamin B_{12} varied.

University of Wisconsin
Madison, Wis.

1033 KITAY E., and SNELL, E. E.: *Some additional nutritional requirements of certain lactic acid bacteria*, J Bact. 60 49-56, July 1950.

In experiments on the nutritive requirements of lactic acid bacteria previously reported not to grow in media of known composition, 18 of the 28 cultures required thymidine, other desoxyribosides, or vitamin B_{12} for growth.

University of Wisconsin
Madison, Wis.

1034 DAVIS, B. D., and MINGIOLI, E. S. *Mutants of Escherichia coli requiring methionine or vitamin B_{12}*. J Bact. 60 17 28, July 1950.

A number of mutants of Escherichia coli have been isolated that require vitamin B_{12} or methionine. They do not respond to homocysteine, the immediate precursor of methionine, and mutants which do respond to homocysteine do not respond to B_{12}. Most of the mutants grow equally rapidly on B_{12} or methionine, but two grow rapidly on methionine and slowly on B_{12}. Vitamin B_{12} appears to be concerned with the methylation of homocysteine. The mutants are stable enough to be used for vitamin B_{12} assay The quantitative response to B_{12} is greater and more prolonged in shaken than in unshaken tubes. With such assays it could be shown that suspensions of wild type Esch. coli rapidly absorb large amounts of vitamin B_{12} from the medium.

Cornell University Medical College
New York, N Y

1035. LANG C. A., and CHOW B F: *Inactivation of microbiological activity of crystalline vitamin B_{12} by reducing agents*, Proc. Soc. Exper Biol. & Med. 75 39-41 Oct. 1950.

When a solution of crystalline vitamin B_{12} was subjected to a series of reducing agents (cysteine, ascorbic acid, thiamine, hydroquinone) between pH 4 and 7 at which range this vitamin is stable, a marked loss of the microbiologic activity occurred. This loss can be attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color The APF activity of the solution was not reduced to the same extent as the microbiologic activity

Johns Hopkins University
Baltimore, Md.

1036. ROBBINS, W J., HERVEY A., and STEBBINS, M. E.: *Studies on Euglena and vitamin B_{12}*, Science 112 455, Oct. 20, 1950.

The use of Euglena gracilis var bacillaris in the bioassay of vitamin B_{12} is reported. It was found that many bacteria and actinomycetes in the soil can synthesize vitamin B_{12}. Yeasts and filamentous fungi apparently are less commonly able to do so. When one part

of fresh soil was shaken with two parts of water the extracts contained amounts of vitamin B_{12} similar to those in cow's milk. The roots of many higher plants contain vitamin B_{12} in amounts ranging from 0.0002 to 0 01 mcg /Gm. of fresh material. It is believed that the vitamin B_{12} is absorbed from soil solutions. A few stems have been shown to contain vitamin B_{12}; leaves and fruits did not. It was found that pond water on which Euglena develops regularly contained vitamin B_{12} throughout the year in concentrations of 0.0001 to 0.001 mcg/cc. The pond mud was shown to contain bacteria and actinomycetes capable of vitamin B_{12} synthesis. The authors believe that the original source of vitamin B_{12} in nature is the synthesizing activity of bacteria and actinomycetes, and not that of higher plants.

New York Botanical Gardens
New York, N Y

1037 WEINBERG E. D *Vitamin requirements of dwarf colony variants of bacteria*, J Infect. Dis. 87 299-306, Nov.-Dec. 1950.

Attempts were made to induce dwarf strains of bacteria to form large colonies. For this purpose certain additions to plain nutrient agar (on which only tiny colonies were produced) were made. Corynebacterium produced large colonies when thiamine was added, Alcaligenes faecalis when thiamine plus vitamin B_{12} was added, Alcaligenes faecalis when reticulogen was added, and Shigella sonnei when yeast extract was added. When again subcultured on plain nutrient agar the cells of large colonies of all the dwarf strains formed only tiny colonies.

University of Chicago
Chicago, Ill.

1038. REGE, D V., and SREENIVASAN A. *Folic acid vitamin B_{12} and nucleic acid synthesis in Lactobacillus casei*, Nature 166 1117 Dec. 30, 1950.

The effects of vitamin B_{12} and folic acid on nucleic acid metabolism were investigated by growing Lactobacillus casei in media containing varying concentrations of these vitamins, singly or together Folic acid was found to enhance the synthesis of desoxyribonucleic acid. This was also true of vitamin B_{12}, although the latter could not completely replace folic acid as a growth factor of L. casei. The most interesting observation, however was that there is a pronounced and more or less all-around additive effect of folic acid and vitamin B_{12}.

University of Bombay
Bombay, India

1039 WOOLLEY D W *Inhibition of synthesis of vitamin B_{12} and of riboflavin by 1,2-dichloro-4,5-diaminobenzene in bacterial cultures*, Proc. Soc. Exper Biol. & Med. 75 745-746, Dec. 1950.

The amounts of vitamin B_{12} and of riboflavin formed in cultures of Bacillus megatherium were decreased by 1,2-dichloro-4,5-diaminobenzene in quantities insufficient to inhibit growth. The production of vitamin B_{12} was stimulated by the dimethyl analog of diaminobenzene. These facts are regarded as compatible with the previously expressed postulates that the dimethyldiamine is a precursor of riboflavin and of vitamin B_{12}, and that the dichloro analog exerts its selective toxicity at least in part, by inhibition of biosynthetical processes in the formation of these vitamins.

Rockefeller Institute for Medical Research
New York, N Y

1040. WOOLLEY D W *Selective toxicity of 1,2-dichloro-4,5-diaminobenzene its relation to requirements for riboflavin and vitamin B_{12}*, J Exper Med 93 13-24, Jan. 1951.

Author's summary: "In a series of 26 species [of organisms] selected from widely differing classes, 1,2 dichloro-4,5-diaminobenzene was toxic to those which did not exhibit a nutritional need for riboflavin plus vitamin B_{12}. It failed to retard the growth of those which needed both of these vitamins. The compound was conceived as an antimetabolite of 1,2-dimethyl-4,5-diaminobenzene. This latter which is contained within the structures of the two vitamins, was pictured as a metabolic precursor of them. It was found to have very slight activity as either riboflavin or as vitamin B_{12} for lactic acid bacteria and algae. The growth-inhibiting action of the dichlorodiaminobenzene was overcome competitively by the dimethyldiaminobenzene, and also, to a lesser extent, by o-phenylenediamine. The toxicity was not influenced by additions of riboflavin plus vitamin B_{12}, except in the cases of two species, where the influence was slight. These facts were considered to support the idea that properly constructed analogs of a precursor of two or more essential participants in cell division may be able to circumvent the counteraction which the vitamin has been found to exert on an antimetabolite of its precursor Alternate explanations of the observed data were likewise considered."

Rockefeller Institute for Medical Research
New York, N Y

1041 WEYGAND F., WACKER, A., and WIRTH, F *Relations between vitamin B_{12}, purines and pyrimidines in connection with Lactobacillus leichmannii 313* Z. Naturforschung 6b 25-34, Jan. Feb. 1951.

The requirement of L. leichmannii for purines and pyrimidines has been determined. For optimal growth only guanine (I) and uracil are needed. In the absence of I or xanthine, adenine (II) inhibits the growth, stimulated by B_{12} or desoxyribosides. It is concluded that the organism can not transform II into I. 4-Amino- and 5-methyl-4-amino-2-thiouracils, benzimidazole and its dimethyl, dibromo and dichloro derivatives also inhibit growth in the absence of II This growth inhibition is competitively antagonized by II and its derivatives and noncompetitively by B_{12}.

1042. SHIVE, W., SIBLEY M. E., and ROGERS, L. L.: *Replacement of vitamin B_{12} by desoxynucleotides in promoting growth of certain lactobacilli*, J Am. Chem. Soc. 73 867-868, Feb. 1951 (in Notes)

The authors state, in part "Although vitamin B_{12} is approximately 10,000 to 30,000 times as effective as the desoxynucleotides in stimulating growth of the lactobacilli in test-tubes, the effect of the desoxynucleotides cannot be accounted for on the basis of conta

with vitamin B_{12}, because the desoxynucleotides are inactive in the Escherichia coli assay are, relative to vitamin B_{12}, more effective in plate assays than in tube assays with the lactobacilli, and migrate differently on paper chromatograms."

University of Texas, and Clayton Foundation for Research Austin, Texas

1043. WOOLLEY D W., and PRINGLE, A. *Structure-activity-relationships among analogs of dimethyldiaminobenzene as growth-inhibitors*, Federation Proc. 10 272, March 1951.

"Because 1,2-dichloro-4,5-diaminobenzene, an analog of 1,2-dimethyl-4,5-diaminobenzene, has been shown to function as an antimetabolite of this precursor of riboflavin and of vitamin B_{12}, a further study of relatives of this metabolite has been undertaken. The dichloro-compound was a selectively toxic agent which affected only species requiring riboflavin and B_{12} nutritionally Furthermore, these 2 vitamins were unable to counteract the analog. Many structural relatives of the dimethyldiamino benzene were synthesized, several of them for the first time, and tested for potency against *Staphylococcus aureus* and mice. Alkylation of one amino-group, or replacement by nitro, abolished activity even when one or both of the methyl-groups was simultaneously exchanged for chlorine atoms. When one hydroxyl replaced one amino, active agents were formed, either when the methyls were untouched, or when they were exchanged for chlorines. Alkylation of the hydroxyl abolished activity The nitrophenols formed by replacing the amino groups with hydroxyl and nitro were very potent, provided that at least one of the methyls was also exchanged for halogen. When the competitive antagonism with the diaminodimethylbenzene was tested, a similar relationship to that already established with analogs of phenylalanine (chloromycetin, etc.) was found. The activity of the aminophenols was only partially reversed by the metabolite, and that of the nitrophenols not at all. Halogenation increased the tendency to non-competitive behaviour with the metabolite. Nevertheless, the amino- and nitro-phenols still actively inhibited the biosynthesis of B_{12}, just as the dichlorodiamine has been shown to do in growing bacterial cultures."

Rockefeller Institute for Medical Research New York, N Y

1044 HENDLIN D., and SOARS, M. H.: *Effect of 5,6-dimethylbenzimidazole and related compounds on the growth of L. lactis Dorner* Federation Proc. 10: 384, March 1951

"5,6-Dimethylbenzimidazole has been reported as a degradation product obtained from vitamin B_{12} on acid hydrolysis. The ability of 5,6-dimethylbenzimidazole and some related compounds to replace vitamin B_{12} in the nutrition of the rat has been described. None of these compounds possessed vitamin B_{12} activity for *Lactobacillus lactis* Dorner when tested at levels as high as 1 mg/ml. More than that, the benzimidazole compounds were found to inhibit markedly the growth of *L. lactis* in the presence of as much as 1 μg/ml. of vitamin B_{12}. The following compounds possessed inhibitory properties 1,2-diamino 4,5-dimethylbenzene, 5-methyl- 4,5-dimethyl and 5,6-dimethylbenzimidazole. No inhibition of growth was obtained with benzimidazole, 1-methyl 2-methyl, 4-methyl-, and 2,5-dimethylbenzimidazole at levels as high as 1 mg/ml. The inhibition obtained with 1,2-diamino, 4,5-dimethylbenzene was competitively antagonized by vitamin B_{12} over a narrow range of inhibitor This was not the case with 5,6-dimethylbenzimidazole. Higher levels of the substituted diaminobenzene produced inhibition which could not be reversed by the known B vitamins, purines, pyrimidines, nucleosides and nucleotides, singly or in combination."

Merck & Co., Inc. Rahway, N J

1045 DAVIS R. L., LAYTON, L. L., and CHOW B. F: *Uptake of radioactive vitamin B_{12} by bacteria in single and mixed cultures*, Federation Proc. 10 380, March 1951

"In view of the lack of agreement as to the role of the intestinal flora with respect to various nutrients in the gut, an in vitro study of vitamin B_{12} metabolism of certain bacteria is being made. Culture media containing vitamin B_{12} labeled with Co^{60} were inoculated with various types of bacteria. After incubation, the resultant bacterial cultures were centrifuged the washed cells and media were assayed for radioactive Co^{60} as $CoSO_4$ and CoS respectively using a Geiger-Muller counter Aliquot samples of media (80 ml.) containing 4 mμ vitamin B_{12} with total activity of 60 cpm were inoculated with *L. leichmannii, E. coli, S lactis R and L. arabinosus*. After 45 hours incubation, the cells were separated, washed and assayed for radioactivity It was found that about one-half of the added activity was present in the first 3 organisms mentioned above and a negligible amount in the last one. Competitive systems of bacteria were also studied the types of organisms cultured were separated by cellophane membranes through which vitamin B_{12} was diffusible. In one such system in which *L. leichmannii* and *L. arabinosus* were separated by cellophane and 6 mμ labeled vitamin B_{12} included in the media, subsequent assay for radioactivity of the grown cells and media demonstrated that *L. leichmannii* absorbed the same proportion of this activity as when cultured alone. Data on the behavior of other competitive bacterial systems for radioactive vitamin B_{12} are also presented as well as methods for the isolation of Co^{60}."

Johns Hopkins University Baltimore, Md.

1046. OGINSKY E. L., SMITH, P H., TONHAZY N E., UMBREIT W W., LICHSTEIN H. C., and CARSON S F *The influence of vitamin B_{12} on oxidation by a mutant strain on Escherichia coli*, J Bact. 61: 581-590, May 1951

The influence of vitamin B_{12} on the rate of oxidation of a number of substrates by resting cell suspensions was studied with a mutant strain of Escherichia coli that required methionine and the vitamin for growth. The vitamin markedly increased the oxidation rate. In the course of the experiments it was found that 36 mcg. of streptomycin per cc. inhibited the oxidation of acetate in the presence or absence of B_{12} when added with the acetate. However streptomycin had no effect on the oxidation if added an hour after the oxidation had begun.

Merck Institute for Therapeutic Research Rahway, N J
Oak Ridge National Laboratories Oak Ridge, Tenn.

1047 LOCHHEAD A. G., and THEXTON R. H.: *Vitamin B_{12} as a growth factor for soil bacteria*, Nature 167 1034 June 23, 1951.

In the course of taxonomic and physiological studies of bacteria incapable of growth in the yeast extract medium, but showing abundant growth upon the addition of soil extract, the ability of vitamin B_{12} to replace soil extract as growth-promoting factor was determined. Of 534 bacterial strains, isolated on a non selective basis from field soil, 75 strains, or 14.0% fell into this special group. Replacement of soil extract by vitamin B_{12} added to give a concentration of 2 mμgm. per ml., permitted optimal growth of 26.7% and submaximal growth of 28.0% of this group. In the case of the remainder vitamin B_{12} gave no growth response.

Department of Agriculture
Ottawa, Canada

1048. McRORIE, R. A., and WILLIAMS W L. *Studies on the relationship between the Lactobacillus bulgaricus factor and pantothenic acid*, J Bact. 61 737 745, June 1951

"All 27 strains of the lactic acid bacteria studied utilize LBF [Lactobacillus bulgaricus factor] under the conditions tested. Twenty five strains appear to require LBF for growth, i.e., LBF is more active for these organisms than an equivalent amount of pantothenate. These include all 12 strains of Lactobacillus bulgaricus tested, 11 out of 11 strains of Lactobacillus acidophilus that grow on the LBF basal medium supplemented with vitamin B_{12}, and the single strain of Lactobacillus delbrueckii tested. For Lactobacillus arabinosus LBF is only one-twelfth as active as would be expected from its pantothenate content, and for Lactobacillus casei the response is approximately equal to the pantothenate content of LBF The yeast, Saccharomyces carlsbergensis, failed to respond to LBF

"The pantothenate fragments, pantoyl lactone and pantoic acid, had no effect on the lactic acid bacteria tested, either alone or in combination with pantothenate. β-Alanine appears to enhance the utilization of pantothenate for these organisms.

"Orotic acid was found to be only stimulatory for all strains of L. bulgaricus except for L. bulgaricus 09 previously reported to require this substance (Wright, 1950b)

"It is suggested that the various forms of LBF are normal intermediates in the biosynthesis of coenzyme A from pantothenic acid."

North Carolina Agricultural Experiment Station
Raleigh, N. C.
Lederle Laboratories
Pearl River N Y

1049 LITTLE, P A., OLESON J J., and WILLIAMS, J H. *Growth studies on Polytomella agilis* Proc. Soc. Exper Biol. & Med. 78 510-513, Nov 1951.

Authors summary: "The nutritional requirements of a strain of Polytomella agilis were investigated. Ethanol, butanol, acetate, propionate or butyrate were utilized as carbon sources. Glutamine was adequate as a sole source of nitrogen. Thiamine and vit. B_{12} were stimulatory A simplified medium, adequate for continuous subculture of this organism, is presented."

Lederle Laboratories
Pearl River N Y

1050. LARDY H A.: *Metabolic functions of the microcatalytic B vitamins* Rec. Chem. Progr 12 9-18 Winter 1951

Biochemists have developed the theory that the water-soluble B vitamins exert their catalytic influence in living cells by being converted to compounds which form the prosthetic or functional group on specific enzymes. Thus, the functional forms for these vitamins are for thiamine, thiamine pyrophosphate (cocarboxylase) for riboflavin, flavin monophosphate and flavin-adenine di nucleotide for niacin, diphosphopyridine nucleotide (cozymase) and triphosphopyridine nucleotide for pyridoxal, pyridoxal phosphate (codecarboxylase) for pantothenic acid, coenzyme A (a pantothenate-containing di nucleotide) For folic acid, citrovorum factor or derivatives, for vitamin B_{12}, and for biotin, the functional forms have not been fully elucidated.

The first five of these vitamins, which function in the metabolic utilization of the major classes of foodstuffs and in the deamination, transamination and decarboxylation of the amino acids, are classed as macro catalysts, and the last three, which function in the synthesis of comparatively small quantities of cellular building blocks, are classed as micro catalysts. Much less is known concerning the structure of the coenzyme form of these micro catalysts, but vitamin B_{12} may already be in the coenzyme form since phosphate is a part of its molecule. All these micro vitamins seem to be involved in the metabolic transformation of compounds containing a single carbon atom. The metabolic functions of biotin and of the folic acid group of vitamins and vitamin B_{12} are discussed.

University of Wisconsin
Madison, Wis.

1051 VILLELA, G G., and ABREU L. A. *Lack of inhibition of growth of Euglena gracilis by vitamin B_{12} oxidation product*, Science 115 205, Feb. 22, 1952.

This experiment shows that the oxidation product resulting when vitamin B_{12} is treated with strong acid and hydrogen peroxide is not a competitive antagonist to vitamin B_{12} when assayed with Euglena gracilis. The antagonism has been shown to exist when Lactobacillus leichmannii 4797 is the test organism.

Instituto Oswaldo Cruz
Rio de Janeiro, Brazil

1052. DAVIS, R. L., LAYTON L. L., and CHOW B. F *Uptake of radioactive vitamin B_{12} by various microorganisms*, Proc. Soc. Exper Biol. & Med. 79 273-276, Feb. 1952.

The incorporation of vitamin B_{12} into bacterial cells of a species requiring this vitamin for growth (Lactobacillus leichmannii) a species which can grow without an exogenous source of this vitamin (L. arabinosus) and one which requires it only during the initial phase of growth (L. lactis Dorner) was studied. Seven other s (Esch. coli, S. Lactis r., B. subtilis, P mirab

mycoides, Esch. freundii, S. dobson) were included in the study. The microorganisms were grown in media containing radioactive vitamin B_{12} tagged with Co^{60}. Radioactivity was found in the cells of all the species studied except L. arabinosus. It was estimated that approximately 8 molecules of the tagged vitamin B_{12} united with each L. leichmannii cell; the number uniting with the cells of the other organisms varied from 12 to 60 and was not related to their growth requirement of vitamin B_{12}; organisms which required no vitamin B_{12} were capable of incorporating it. No radioactivity appeared in the cells when radioactive cobaltous chloride was used instead of radioactive vitamin B_{12}. To investigate the fate of the tagged vitamin in a system of competitive organisms such as is found in the intestinal tract, two microorganisms were separated by a cellophane membrane which was readily permeable to the vitamin; radioactivity entered the cells of both organisms in essentially the same amounts as when each species was grown alone.

Johns Hopkins University
Baltimore, Md.

1053 OGINSKY E. *Uptake of vitamin B_{12} by Escherichia coli*, Arch. Biochem. & Biophysics 36: 71-79 March 1952 (abstr Blood 7: 857 Aug. 1952)

"Cells of a vitamin B_{12} deficient mutant strain of E. coli are capable of absorbing radioactive vitamin B_{12}, labeled with Co^{60} in excess of amounts required for growth or oxidation. This observation agrees with the finding of Koditschek and Hendlin using Lactobacillus lactis that these bacteria take up considerably more vitamin than is required for growth.

"In the presence of glucose, the absorption rate of deficient cells was decreased. No exchange of intracellular B_{12} in respiring cells could be demonstrated."

Merck Institute for Therapeutic Research
Rahway, N. J.

1054 DAVIS, R. L., and CHOW B. F.: *Determination of vitamin B_{12} by means of its combination with L. leichmannii resting cells*, Federation Proc. 11: 466, March 1952.

"Both the growing and 'resting' cell of *L. leichmannii* (ATCC 4947) can incorporate vitamin B_{12} from the environment; the latter in greater magnitude. These findings enabled us to develop some techniques useful in the rapid concentration and estimation of both radioactive and non-radioactive vitamin B_{12} present in a variety of biological fluids. This was accomplished by the addition of a suspension of 'resting' cells of *L. leichmannii* to given volumes of the test fluid. After standing at room temperature for approximately 30 minutes the microorganisms, which by then would have taken up quantitatively the vitamin B_{12}, were centrifuged and washed with isotonic saline solution. With samples containing radioactive vitamin B_{12}, the washed organisms were transferred to a planchet and the radioactivity measured. To estimate the non-radioactive vitamin B_{12}, two quantitative procedures were developed. 1) The microbiological assay in which the washed cells were suspended in normal saline solution and heated at 60 C. for 1 hour and the resulting suspension was assayed microbiologically for vitamin B_{12}; 2) an isotope-dilution method, in which use was made of a standard curve relating the uptake of radioactivity and the total quantity of vitamin B_{12} consisting of a constant amount of radioactive and increasing amounts of non-radioactive vitamin B_{12}. The uptake of vitamin B_{12} by *L. leichmannii* was effective over a wide range of pH's but was diminished at salt concentration above isotonicity. The advantages of our procedure lie in its rapidity, ease and comparative lack of inhibitors."

Johns Hopkins University
Baltimore, Md.

1055. DUBNOFF J. W. *Role of B_{12} in methionine synthesis in E. coli mutant 113-3*, Federation Proc. 11: 205, March 1952.

"Homocysteine, glutathione or cysteine partially replaces the B_{12} requirement of the *E. coli* mutant 113-3. The growth promoting ability of these compounds is markedly enhanced by small amounts of B_{12}. This second requirement for B_{12} is at least partially replaced by the specific methyl donor, dimethyl-β-propiothetin provided homocysteine or its thiolactone is used to replace the major B_{12} requirement. Other methyl compounds such as choline, betaine, dimethylthetin, and dimethyl-γ-butyrothetin which are active in animals are completely inactive in this mutant."

California Institute of Technology
Pasadena, Calif.

1056. ROBBINS W. J., HERVEY A., and STEBBINS M. E. *Bacteria in air make vitamin B-12 from dust*, Science News Letter 62: 296, Nov. 8, 1952.

Bacteria in the air can synthesize vitamin B_{12} from dust. This was shown when pure water was first exposed to air for two months and then seeded with Euglena. Euglena are one-celled green plants that grow only in the presence of vitamin B_{12}.

"As the Euglena thrived, ... [the investigators] deduced that B-12 was present. They were able to measure the vitamin content of the water by the amount of Euglena growth, and found two millionths of a millionth of a gram (a micro-micro-gram) of the vitamin in each milli-liter of water.

"As the water was completely sterile and pure at the beginning of the experiment, the scientists were able to deduce that bacteria from the air which fell into the water had converted food material present as dust in the air into the vitamin."

New York Botanical Garden
New York, N. Y.

1057 DAVIS, R. L., and CHOW B. F.: *Compounds inhibiting uptake of vitamin B_{12} by "resting" bacterial cells*, Federation Proc. 12: 440, March 1953.

"Compounds such as nucleic acids, mucopolysaccharides and others can reduce the uptake of environmental vitamin B_{12} by the 'resting' cells of Lactobacillus leichmannii ATCC 4792(LL). The extent of this inhibition of uptake is dependent on the quantity of the test substance, the chemical structure, in some instances the polymerized state, and finally on the processing of the 'resting'

cells. The order of addition is likewise of importance: the most effective sequence being the test substance, resting cells, and vitamin B_{12}. The most inhibitory substances, heparin and other sulfonated compounds are capable of reducing by 50% the uptake of 20 mγ vitamin B_{12} in 2-5 mcg. quantities, in a system containing approximately 2×10^9 resting cells. Two strains of *Escherichia coli*, namely 113-2 and Waksman, whose uptake for vitamin B_{12} was demonstrated by B. D Davis and Oginsky responded differently than LL., qualitatively and quantitatively to these compounds. For example, the uptake of vitamin B_{12} by both strains of *E coli* was unchanged by heparin. Methylene blue, laurylethyleneoxide and trisodiumethylenediaminetetraacetate were inhibitory only to *E coli*, whereas neomycin, polyglutamic acid (M.W 8000) and 'hyamine were inhibitory to all. Since consistent and reproducible responses to the inhibitors were noted, a satisfactory procedure was established to measure these compounds in unknown samples by comparing with a standard curve, the amount of reduction of a given dose of vitamin B_{12}. Other applications and implications on these observations will be reviewed."

Johns Hopkins University
Baltimore, Md.

1058. DUBNOFF J W: *Influence of vitamin B_{12} on enzyme activity in E coli 113-3* Federation Proc. 12 198, March 1953.

"The influence of B_{12} on the oxidation of a wide variety of substrates first demonstrated by Oginsky *et al.* is independent of the cytochrome system since the oxidation measured by the reduction of methylene blue and tetrazolium is similarly affected. Certain preparations of the mutant which do not show the B_{12} effect will respond to the vitamin if reduced glutathione is added. Cysteine will not replace glutathione. The aging' process usually required to show a response to B_{12} results in a decrease in enzymatic activity Data will be presented suggesting that this decrease in activity is related to a decrease in protein sulfhydryl as measured by the ferricyanide method and that reduced glutathione, B_{12} and certain substrates form a system that increases protein sulfhydryl in this mutant."

California Institute of Technology
Pasadena, Calif.

1059 KALAN E., and CEITHAML, J *Synthesis of methionine in E. coli,* Federation Proc. 12: 228 March 1953.

By the use of biochemical mutants, vitamin B_{12} has been implicated in the biosynthesis of methionine in *E coli* W ATCC 9637 (DAVIS AND MINGIOLI, *J* Bact. 60 17 1950) [*See* Abstr 1034] A series of methionine requiring mutants has been isolated in our laboratory These were studied on agar plates which contained the minimal medium supplemented with methionine or various possible methionine precursors, individually, and in combination with vitamin B_{12}. It was found that these mutants could be classified into four main categories. The mutants in the first category grow only in the presence of methionine. Those in the second group require either methionine or vitamin B_{12} for growth. The third category consists of those mutants which exhibit optimal growth in the presence of the following supplements methionine, homocysteine, homocystine or cystathionine. Addition of B_{12} effects no stimulation of growth. The mutants of the fourth group are similar in their requirements to those of the third group. Moreover some of these mutants can grow on alanine, homoserine, valine, isoleucine or alpha amino butyric acid. However optimal growth occurs only in the case of methionine. On the other effective supplements, growth is slight, but when B_{12} is added, growth is stimulated. These results support the view that B_{12} is involved in methionine synthesis. They also indicate possible amino acid precursors for the 4-carbon moiety of the methionine molecule."

University of Chicago
Chicago, Ill.

ASSAYS OF VITAMIN B_{12} ACTIVITY AND CONTENT

CHROMATOGRAPHIC

1060. TISHKOFF G. H., ZAFFARONI, A., and TESLUK, H.: *Purified liver extract chemical nature as determined by paper partition chromatography* J Biol. Chem. 175 857-862, Sept. 1948.

Studies of a liver extract containing a high concentration of the anti pernicious anemia factor revealed the presence of one or more polypeptides of high molecular weight, some free amino acids and riboflavin. Folic acid and xanthopterin were not detected. The polypeptide which is presumably the active constituent, was separated from the free amino acids and the amino acids obtained by hydrolysis of the polypeptide were identified by paper chromatography

1061. WINSTEN W A., and EIGEN E.: *Paper chromatography of vitamin B_{12} and related bacterial growth factors,* Federation Proc. 8: 265-266, March 1949; J Biol. Chem. 177 989-990, Feb. 1949

The paper chromatographic procedure for separating the six alternate growth factors for Lactobacillus leichmannii, two of which are found in vitamin B_{12} concentrates, is reported, as well as the method of recognizing the positions of the several growth factors on a chromatogram by the use of L. leichmannii as a microbiologic indicator The slow movement of the two forms of vitamin B_{12} on a paper chromatogram with n-butanol as the mobile phase permits the separation of these entities from the faster moving substitute growth factors. The isolated vitamin B_{12} entities may then be assayed by the usual tube assay procedure.

1062. YACOWITZ, H., NORRIS L. C., and HEUSER, G. F *An improved method for the microbiological assay of growth factors on paper chromatograms,* Proc. Soc. Exper Biol. & Med. 71 372-374, July 1949

A modification of the method of Winsten and Eigen (Abstr 1061) for the microbiologic assay of nutritional growth factor is described. This modification permits

both qualitative and quantitative assay by means of paper chromatograms, and avoids the interference of thymidine in the assay of vitamin B_{12}. This method has been found to be sensitive to 0.1 millimicrogram of vitamin B_{12} present in the developed chromatogram. Lactobacillus leichmannii is used as the assay organism.

1063 BORSOOK, H., DEASY C. L., HAAGEN-SMIT A. J., KEIGHLEY G., and LOWY P H. *A convenient quick method of obtaining vitamin B_{12} concentrate*, Science 110 528-529 Nov 18, 1949

The nonprotein filtrate of liver homogenate (proteins coagulated by boiling at pH 5) chromatographed on starch columns gave a reddish brown fraction in the first portions of the effluent. The method offers a way of obtaining B_{12}-active material from commercially available sources, which already contain the activity in a conveniently small volume.

California Institute of Technology
Pasadena, Calif.

1064 WINSTEN W A., and EIGEN, E. *Paper chromatography of vitamin B_{12} and related bacterial growth factors*, J Biol. Chem. 181 109 120, Nov 1949

A paper chromatographic procedure for separating vitamin B_{12} and related growth factors is described. In addition to vitamin B_{12}, five other substitute growth factors of L. leichmannii 313 have been recognized. They are desoxyribosides.

1065. WIJMENGA, H. G., LENS, J., and MIDDELBEEK, A. *Some properties of vitamin B_{12}*, Chem. Weekblad. 45: 342-343, 1949 (abstr C. A. 43 9193f Nov 25, 1949)

"Cryst. vitamin B_{12} (I) (yield, 25 mg. per ton liver) had 3.8 ± 0.4% Co and absorption coeff. (log ε % cm.) 280 mμ, 2.11 360 mμ, 2.26. Impure I contg. 0.45 ± 0.04% Co had absorption coeff. 280 mμ, 2.20 360 mμ, 2.40 550 mμ, 1.86 detd. with a Beckman spectrophotometer The following values were obtained with a Kipp instrument using the same soln. 80 mμ, 2.44 360 mμ, 2.50 510 mμ, 2.12. The observation that impure I contg. but 0.45% Co has absorption max. at the same wave length as pure I, and that the impure prepn. has a higher absorption coeff. than pure I indicates that the abs. coeff.-wave length curve cannot be used as an index of purity of I. In contrast with the findings of Rickes, *et al.*, a 50% soln. of pure I in 0.1 *N* HCl was stable, as shown by no change in the absorption spectrum and no liberation of free Co, on 8 days standing. The unchanged absorption spectrum is in contrast with the report of E. Lester Smith but this variance may be due to the still inexplicable result obtained in the following expts. A soln. of I was aerated for 1.5 hrs. At wave lengths below 300 mμ a total end absorption occurred and the absorption max. at 280 mμ had disappeared, without affecting the absorption at 360 mμ. Chromatographic absorption of the aerated I on neutral Al_2O_3, gave 2 fractions, each of which had absorption max. at 280 mμ. This was attributed to oxidation until the same result was obtained by passing CO_2 through the soln. Heating I in distd. H_2O (pH 5.3) results in formation of a yellow-brown color"

1066. SHAW G E. *Paper chromatography of liver extracts*, Biochem. J 44 liv 1949

A paper chromatographic method for the assay of vitamin B_{12} is described.

1067 CUTHBERTSON, W F J., and SMITH, E. L. *Chromatography of the vitamin B_{12} group of factors*, Biochem. J 44 v-vi, 1949

"The two red substances present in liver extract (one probably identical with vitamin B_{12}) thymidine and a fourth unidentified factor can be demonstrated by a combination of partition chromatography on paper with microbiological assay on a solid medium."

1068. SMITH E. L., and CUTHBERTSON W F J *Paper chromatography of the vitamin B_{12} group of factors* Biochem. J 45 xii, 1949 (in Soc. Proc.)

All five naturally occurring purine and pyrimidine desoxyribosides show growth activity with L. lactis Dorner and their presence in extracts from liver and from Streptomyces griseus interferes with the plate assay process for vitamin B_{12}. A method of separating them by paper chromatography prior to B_{12} assay is indicated.

1069 SMITH, E. L., CUTHBERTSON W F J., WALKER, A., and LEES K. A.: *Partition chromatography of the vitamin B_{12} group of factors*, Federation Proc. 9 230, March 1950.

1070. WOODRUFF H. B., and FOSTER, J C.: *Analysis for vitamin B_{12} and vitamin B_{12a} by paper strip chromatography* J Biol. Chem. 183 569-576, April 1950.

Authors summary: "A method has been presented for quantitative estimation of vitamins B_{12} and B_{12a}, combining microbiological assay and paper strip chromatography A small corrective factor is required to compensate for conversion of vitamin B_{12} to vitamin B_{12a} which may take place during the analysis. The method has been applied to microbiological fermentation broths and to injectable liver extracts (U.S.P) for the analysis for vitamin B_{12} type materials. No quantitative relationship was found between the amount of these vitamins contained in the samples and the Lactobacillus lactis titrimetic assay of the samples."

Merck & C , Inc.
Rahway N J

1071 FANTES K. H., IRELAND D M., and GREEN N.: *A colorimetric assay method for vitamin B_{12}*, Biochem. J 46 xxxiv xxxv May 1950 (in Soc. Proc.)

A colorimetric assay for vitamin B_{12} is described which is capable of measuring about 40 mcg. in not more than 2 cc. Related cobalt-containing factors of the vitamin B_{12} complex, such as vitamin B_{12b} behave like vitamin B_{12} itself in this procedure.

Glaxo Laboratories
Greenford and Barnard Castle
England

1072. CHARGAFF E., LEVINE, C., GREEN C., and KREAM J C.: *Study of some constituents of vitamin B_{12}*, Experientia 6 229-231 June 1950

The occurrence of nucleic acids and the content of the amino compound in vitamin B_{12} has been studied by paper chromatography Neither purine nor pyrimidine or desoxyribose was found to be present. The amino derivative (calculated as 2-amino-1-propanol) was found to be present in a proportion of 2 mols per one mol of phosphorus.

Columbia University
New York, N. Y.

1073. PICKEN J C., JR., and BAURIEDEL, W R.: *Comparative bioautography of vitamin B_{12} and related growth factors using Euglena gracilis and Lactobacillus leichmannii*, Proc. Soc. Exper Biol. & Med. 75 511-515, Nov 1950.

A number of preparations to which Euglena gracilis or Lactobacillus leichmannii has been reported to give a quantitative growth response (crystalline vitamin B_{12}, liver extract, corn steep liquor desoxyribonucleic acid) were subjected, in duplicate, to paper chromatography in order to separate different factors. One of each pair of paper strips was then placed in contact for about five minutes with an agar plate seeded with E. gracilis and the other with a plate seeded with L. leichmannii. The plates were then incubated, and the growth response of the two organisms on different concentrations of the tested preparations was compared by means of photographs and graphs (bioautographs) The least amount of crystalline vitamin B_{12} that would give definite visible growth when applied to the plate on a 1 cm^2 piece of filter paper was 0.01 mcg. for E. gracilis and approximately 0.5 mcg. for L. leichmannii. E. gracilis responded only in the area of the slower moving factors of the liver extract, where vitamin B_{12} is expected to appear while L. leichmannii responded to three more rapidly moving factors in addition to the slow-moving factor There were indications that the fastest moving factor was thymidine. E. gracilis did not respond to corn steep liquor while L. leichmannii responded to four moving factors. E. gracilis did not respond to any of the desoxyribonucleic acid preparations unless vitamin B_{12} was added, in which case growth occurred in the area of the slow moving factors. L. leichmannii demonstrated the existence of a slow-moving factor or factors in the untreated desoxyribonucleic acid preparations. Since these factors promoted the growth of one organism but not of the other they cannot be considered identical with crystalline vitamin B_{12}. Thus the comparative bioautograph method may be useful in studying the slow-moving factors, as a qualitative method of indicating activities present in preparations which are to be assayed for vitamin B_{12}, and as a guide in separation and purification procedures.

Iowa State College
Ames, Iowa

1074 KOCHER, V., KARRER, R., and MÜLLER, H. R. *Paper chromatography of vitamin B_{12} and desoxyribonucleosides with phenol, n-butanol and collidin*, Z. Vitaminforsch. 21 403-409 1950.

St. Jakob Microbiological Laboratory
Switzerland

1075 VEER W L. C., EDELHAUSEN J H., WIJMENGA H G., and LENS, J: *Vitamin B_{12}. I The relation between vitamin B_{12} and B_{12b}*. Biochim. Biophys. Acta 6: 225-228, 1950

The spectrum of vitamin B_{12} remains unchanged in mildly acid solution if kept in the dark; on exposure to light it is transformed into B_{12b}. An irradiated solution of B_{12b} (pH—4) when kept in the dark, shows slow reversal to B_{12}. Addition of KCN to B_{12b} effects rapid conversion to B_{12}. B_{12} is either a cyan complex or it has a spectrum which is identical with a B_{12b}-cyan complex.

1076. WIJMENGA, H. G., VEER, W L. C., and LENS J *Vitamin B_{12}. II The influence of HCN on some factors of the vitamin B_{12} group*, Biochim. Biophys. Acta 6 229-236, 1950.

The absorption spectra (visible and ultraviolet) of vitamin B_{12a} and B_{12b} are identical and the two forms cannot be distinguished by chromatography (n-butanol solvent)

1077 HOLDSWORTH, E. S., and FORD J E.: *Differentiation of vitamin B_{12} active compounds by ionophoresis and microbiological assay* Nature 171 148-150, 1953 (abstr Blood 8 487 May 1953)

"There are several compounds related to vitamin B_{12} but not in the cobalamin series. Using paper chromatography factor A, vitamin B_{12b} and pseudo-vitamins B_{12} and B_{12f} could not be separated with the solvents usually employed. The ionophoresis of these substances was tested. Results with veronal/acetate buffer were not helpful, but where 0.5 N acetic acid was used, with 0.01 per cent potassium cyanide, five substances could be distinguished. These, with their mobilities expressed as cm^2V^{-1} sec^{-1} were factor A (vitamin B_{12m}) 3.9×10^{-5}; factor B, 5.0×10^{-5} factor C, -1.4×10^{-5} pseudo-vitamin B_{12}, 1.5×10^{-5} vitamin B_{12}, zero mobility

"The growth activities of these and other factors for micro-organisms were compared, B. coli (tube assay) B coli (plate assay) and L. leichmannii being used. Factor A and vitamin B_{12m} have similar properties, as have pseudo-vitamins B_{12} and B_{12f}. The two groups differ markedly in microbiologic activities and there are further differences with factors B, WR, and C. Factor WR is probably a mixture that includes inhibitors for the growth of B coli"

University of Reading
Reading, England

VALENCY; MAGNETIC PROPERTIES

1078. LALAND P., and CLOSS K. *Formation of trivalent cobalt complexes in protein hydrolysates* Nature 163 565, April 9 1949 (Letter to Editor)

1079 GRÜN F., and MENASSE, R.: *Determination of the magnetic susceptibility of vitamin B_{12}*, Experientia 6 263-264, July 15, 1950.

Measurements of the magnetic susceptib min B_{12} have shown it to be diamagnetic.

Microbiologic measurement of the vitamin B_{12} activity of seven samples of liver extracts for oral administration showed a wide variation in activity. The daily dose of the liver extracts is 45 cc., and represents a vitamin B_{12} activity equivalent to 30 to 140 mcg. per day. This is considerably more than the 5 mcg. doses of crystalline vitamin B_{12} which have been found to be effective when given orally together with gastric juice to pernicious anemia patients.

S. E. Massengill Co.
Bristol, Tenn.

1098. TRENNER, N. R., BUHS, R. P., BACHER, F. A., and GAKENHEIMER, W. C.: *A note concerning the incompatibility of vitamin B_{12} and ascorbic acid*, J. Am. Pharm. A. (Sc. Ed.) 39: 361, June 1950.

The incompatibility of mixtures of vitamin B_{12} and ascorbic acid has been reported. This incompatibility has been investigated further. The existence of B_{12} analogs possibly has considerable significance in relation to the compatibility of certain vitamin B_{12} concentrates and ascorbic acid. Highly purified crystalline vitamin B_{12} preparations show a much higher degree of stability in the presence of pure ascorbic acid than previously reported.

Examination of a number of commercial vitamin B_{12} preparations for ascorbic acid compatibility revealed that they vary greatly in this respect. These results are interpreted as evidence of the presence, in varying amount of ascorbic acid–incompatible analogs of vitamin B_{12} in some commercial preparations.

Merck & Co., Inc.
Rahway, N. J.

1099. HARTLEY, F., STROSS, P., and STUCKEY, R. E.: *Some pharmaceutical aspects of vitamin B_{12}*, J. Pharm. and Pharmacol. 2: 648-659, Oct. 1950.

1100. HERRNRING, G.: *Quantitative method for determination of vitamin B_{12} content of commercial liver preparations*, Folia haematol. 70: 110-1950.

1101. BOXER, G. E., and RICKARDS, J. C.: *Chemical determination of vitamin B_{12}. IV. Assay of vitamin B_{12} in multivitamin preparations and biological materials*, Arch. Biochem. 30: 392-401, Feb. 1951.

The colorimetric method for determining vitamin B_{12} (cyanocobalamin) has been applied to certain multivitamin preparations, liver and microbial concentrates, urine, gastric juice, and aqueous liver extracts. The determination of cyanide released after illumination with visible light proved to be specific for vitamin B_{12} in the substrates investigated. The daily excretion of cyanide and vitamin B_{12} in the urine was determined in 2 subjects. Cyanide excretion was rather constant and did not increase when a large dose of vitamin B_{12} was injected. Vitamin B_{12} excretion was irregular but some of the vitamin was found at all times in the urine. There was prompt excretion of a large injected dose of vitamin B_{12}, indicating that there was no need of, or facilities for the storage of the vitamin.

Merck & Co., Inc.
Rahway, N. J.

1102. BOXER, G. E.: *Chemical assay method for vitamin B_{12}*, Science 113: 153, Feb. 9, 1951.

At the AAAS symposium on "Recent Advances in the Chemistry of the Antibiotics and Vitamins," a chemical method for assay of vitamin B_{12} was described. The method depends on the liberation by vitamin B_{12} of one mole of cyanide, a reaction which is accelerated by light. The cyanide is determined by a millimicro method that is sufficiently sensitive to allow the determination of vitamin B_{12} in urine and tissues.

Merck & Co., Inc.
Rahway, N. J.

1103. PRITCHARD, H.: *Experiences with the microbiological assay of vitamin B_{12} in an analytical and consulting laboratory*, Analyst 76: 155-160, March 1951.

The tube method of microbiological assay of vitamin B_{12} with Lactobacillus leichmannii 313 has been adapted for routine use in a laboratory where demands for the assay are only intermittent. The vitamin B_{12} potency has been given for a number of materials used in the compounding trade as determined by this test.

1104. MARSH, M. M., and KUZEL, N. R.: *Separation and determination of crystalline vitamin B_{12} in synthetic vitamin mixtures*, Anal. Chem. 23: 1773-1776, Dec. 1951.

Substances which interfere in the spectrophotometric determination of vitamin B_{12} can be satisfactorily removed by mixed cation and anion exchange resin columns.

1105. ROSENBLUM, C., and WOODBURY, D. T.: *Determination of the stability of vitamin B_{12} in multivitamin mixture by a radioactive indicator method*, J. Am. Pharm. A. (Sc. Ed.) 41: 368-371, July 1952.

Authors summary: "1. The stability of vitamin B_{12} in four different multivitamin capsule materials, after storage at room temperature for more than thirteen months, was determined by means of cobalt 60 labeled vitamin as radioactive tracer. 2. The tracer vitamin was extracted by a procedure involving three separate solvents. 3. The quantity of B_{12} extracted was determined by its radioactivity rather than by conventional chemical assays, and the identification of the radioactivity with the vitamin was made by determining its distribution in the system water–benzyl alcohol. 4. The maximum losses noted ranged between 9 per cent and 31 per cent in more than a year. The valence of the iron (ferrous or ferric) present in the capsule materials had no effect on the stability."

Merck & Co., Inc.
Rahway, N. J.

1106. HEINLE, R. W., BETHELL, F. H., CASTLE, W. B., LONDON, I. M., and SALTER, W. T.: *Control of U. S. P. anti-anemia preparations: special report of the United States Pharmacopeia Anti-anemia Preparations Advisory Board*, J.A.M.A. 151: 40-43, Jan. 3, 1953.

Preparations for pernicious anemia are evaluated as of this date. These include vitamin B_{12}, liver extracts, and folic acid. The role of intrinsic factor is discussed.

1107 TAUB, A., and LIEBERMAN H.: *Stability of vitamin B_{12}—folic acid parenteral solutions* J Am. Pharm. A. (Sc. Ed.) 42: 183-186, April 1953.

"The low solubility of folic acid in water except at a relatively high pH, has prevented its use in parenteral solutions in combination with vitamin B_{12} (cyanocobalamin) which attains optimum stability in the pH range 4 to 6.5. The present study utilizes selected solubilizers for folic acid, including aminoacetic acid, methyl glucamine, and niacinamide within a pH range of 6 to 6.5. Results indicate that niacinamide is the best of the solubilizers studied. By special processing it is possible to dissolve and maintain in solution 5 mg. of folic acid per cc. at pH 6 under conditions compatible with crystalline vitamin B_{12} at a concentration of 30 µg. per cc. Solutions show no significant loss of potency of either the folic acid or the vitamin B_{12} after storage at room temperature and under accelerated conditions, and display satisfactory stability with respect to clarity of solution."

Columbia University College of Pharmacy
New York, N Y

1108. CAMPBELL, J A., McLAUGHLAN J M., CLARK, J A., and DUNNETT C. W *The six point design in the U S P microbiological assay of vitamin B_{12}*, J Am. Pharm. A. (Sc. Ed.) 42 276-283, May 1953.

"Since few published data were available concerning the precision of the U.S.P method for [assaying] Vitamin B_{12}, a detailed statistical study was undertaken to assess the importance of factors affecting the response. On the basis of a linear relation between the logarithm of dose and the logarithm of response, the six point design (three doses of standard and three doses of unknown) was applied to this assay The design was found to have several advantages, and under well-controlled conditions it may be simplified still further The method was precise and reproducible. The linear relationship between log dose and log response was valid, and was not influenced by the type of product being assayed, e.g., liver extract injectable or vitamin B_{12} concentrate. The response to vitamin B_{12} was influenced by the preparation of tubes and by their position during autoclaving and incubation. To avoid possible bias caused by these factors, the tubes were randomized before addition of the media."

Food and Drug Laboratories
Department of National Health and Welfare
Ottawa, Canada

Animal Tissues

1109 REGISTER, U D., LEWIS, U J., THOMPSON H. T., and ELVEHJEM, C. A.: *Variations in the vitamin B_{12} content of selected samples of pork and beef muscle*, Proc. Soc. Exper Biol. & Med. 70 167 168, Jan. 1949

It has been found that vitamin B_{12} will give a quantitative response to an assay which has been used for a growth factor in liver preparations. This procedure consists of placing rats on a basal diet for a two-week depletion period and then following the growth response during two weeks when the material to be tested is given.

The vitamin B_{12} activity of pork and beef muscle was tested by this method, using an assayed liver preparation as a standard. The samples of beef were found to contain minimum values of 2 mcg. of vitamin B_{12} per 100 Gm.; pork from a sow that lactated normally contained 1 mcg. per 100 Gm. and pork from a sow with abnormal lactation (her young died soon after birth) contained only a trace. The wide variation in pork samples may be due to the fact that swine are monogastric animals and therefore may not be supplied with ample quantities of vitamin B_{12} and possibly other factors that can be produced by microorganisms in the rumen of cattle.

1110 REGISTER, U D., RUEGAMER, W R., and ELVEHJEM, C. A. *An improved assay for growth factor in liver extracts*, J Biol. Chem. 177 129-134, Jan. 1949

Rats are fed a diet of yellow corn, soybean meal with iodinated casein and vitamins. Injections of liver extract *during 1 or 2 weeks give quantitative growth responses.* The method proved satisfactory for commercial liver extracts and may also be suitable for the estimation of vitamin B_{12}.

1111 FROST D V., FRICKE, H. H., and SPRUTH, H. C. *Rat growth assay for vitamin B_{12}*. Proc. Soc. Exper Biol. & Med. 72 102-106, Oct. 1949

A method of assaying vitamin B_{12} by means of rat growth is described. The experimental animals were depleted of vitamin B_{12} by keeping them on a diet of purified casein for 7 to 14 days (usually 7 since longer depletion increased the mortality) The response of these animals to three vitamin B_{12} preparations—Cobione, a concentrate containing 25 mcg. of vitamin B_{12} per cc., and a concentrate containing any desired concentration of vitamin B_{12}—was then determined. Growth was found to be proportional to the amount of vitamin B_{12} administered in the critical range of 0.025 to 0.1 mcg. per rat per day Oral administration at these levels gave approximately the same response as injection. Addition of the vitamin to the diet, multiple dosing, and single dosing on the first day of assay also gave roughly equivalent results. Two liver extracts showed correlation between their effectiveness in rat growth and in their vitamin B_{12} content, as determined by microbiologic assay

Abbott Research Laboratories
North Chicago, Ill.

1112. SAUBERLICH, H. E.: *The effect of folic acid upon the urinary excretion of the growth factor required by Leuconostoc citrovorum*, J Biol. Chem. 181 467-473, Dec. 1949

Urinary excretion of Leuconostoc citrovorum factor of rats on a diet unsupplemented by folic acid was low The urine of rats on a folic acid supplemented diet (1 to 10 mcg./Gm.) was high in citrovorum factor the activity being proportional to the amount of folic acid ingested. Sulfaguanidine suppressed only slightly the excretion of citrovorum factor vitamin B_{12} or ascorbic acid did not increase it. The ingestion of folic aci creased the urinary excretion also in human subj

1113 COATES, M. E., HARRISON G. F., and KON S. K. *The measurement of vitamin B_{12} by biological assay with chicks* Biochem, J 46 vii-viii, Feb. 1950 (In Soc. Proc.)

For this assay, newly hatched chicks from hens depleted of vitamin B_{12} were used. They showed signs of deficiency of vitamin B_{12} at 4 weeks of age. These depleted chicks responded equally to microbiologically equivalent doses of vitamin B_{12} in crystalline form, in Examen, and in crude liver extract. Using Examen as the source of vitamin B_{12}, a linear relationship was found at dose levels of vitamin B_{12} of 1 to 3 mcg./100 Gm. diet between body weight at 4 weeks and the logarithm of the dose. This test proved applicable to the assay of natural products although the limits of error were large. The values obtained for two samples of fish solubles were much higher than those obtained microbiologically by other investigators.

National Institute for Research in Dairying, University of Reading, and Agricultural Research Council England

1114 TAPPAN D V., LEWIS, U J., REGISTER, U D., and ELVEHJEM, C. A.: *Further studies on the rat growth assay for vitamin B_{12} activity* Arch. Biochem. 29: 408-412, Dec. 1950.

Authors summary: "The values for vitamin B_{12} activity obtained by the rat assay method correspond very closely with those obtained using a microbiological procedure. Folic acid has no observable influence on rats receiving the assay ration while cobalt affects the assay only if it is fed at a level high enough to cause a growth depression. Vitamin C exerts an inconsistent but probable growth-promoting effect in the assay Under the conditions of these experiments aureomycin causes a marked increase in the growth of rats, even in the presence of vitamin B_{12}."

1115. BLACK, D J G., GETTY J., COATES, M. E., HARRISON G. F., and KON, S. K.: *Hatchability of eggs and the production of chicks for the assay of vitamin B_{12}*. Biochem. J 46 viii, 1950.

1116. COATES, M. E., HARRISON, G F., and KON S. K.: *The chick assay of vitamin B_{12} and the animal protein factor* Analyst 76 146-150, March 1951

A method of assay with chicks for vitamin B_{12} and the animal protein factor is described and its accuracy discussed. Evidence is presented for the existence of other factors besides vitamin B_{12} in the animal protein factor Difficulty in obtaining satisfactory responses to crystalline vitamin B_{12} is reported.

1117 SCHWEIGERT B. S., SCHEID H. E., and MARQUETTE, M. M.: *Determination of vitamin B_{12} in animal tissues*, Federation Proc. 10: 394, March 1951

"Experiments on the effect of adding reducing agents and the method of sterilization of the samples and medium were conducted *to improve* the method of determining vitamin B_{12} with *L. leichmannii* as the test organism. The results obtained when the samples and medium were autoclaved for 10 minutes in the presence of 2 mg. of thioglycollic acid per tube (10 ml. total volume) were comparable to those obtained after Seitz filtration. The activity of several enzyme preparations (trypsin, pancreatin, takadiastase, chick pancreas and bacterial preparations) was investigated for the release of microbiologically unavailable forms of vitamin B_{12} in animal tissues. The values obtained for muscle and organ meats were approximately doubled after these treatments as compared to the values obtained for cold water extracts. The vitamin B_{12} potency of some of these samples was also determined by the rat and chick assay The range in vitamin B_{12} potency (expressed as μg/100-gm. fresh weight) determined by the microbiological and animal assays was as follows beef round, 1.5-2.5 pork loin and ham, 1 0-2.0; lamb leg, 2.0-4.0 beef kidney 15-30 and beef liver 30-100. The vitamin B_{12} content of the rat livers determined microbiologically was increased as the amount of vitamin B_{12} fed was increased. The vitamin B_{12} potency of the meat samples calculated from the data on the vitamin B_{12} content of the rat livers was in accord with the potency calculated from the growth data."

1118. COOPERMAN J M., and TABENKIN B.: *Vitamin B_{12} activity of 5,6-dimethylbenzimidazole, 1,2 diamino-4,5-dimethylbenzene and riboflavin for the rat*, Federation Proc. 10: 175, March 1951.

"Rats maintained on the vitamin B_{12} depletion diets of Register *et al.* (*J Biol. Chem.* 177 129 1949) and modified so that 100 mg. crystalline thyroxine per kg. replaced the iodinated casein, and of Emerson (*Proc. Soc. Exper Biol. & Med.* 70: 392, 1949) were given 5,6-dimethylbenzimidazole (*A*) 1,2-diamino-4,5-dimethylbenzene (*B*) and riboflavin (*C*) to determine the vitamin B_{12}-like activity of these compounds. When administered intraperitoneally *compound B* had a very low order of activity Under these conditions *compounds A* and *C* can partially satisfy the vitamin B_{12} requirement. Ten or 15 rats were used in each group. These results are shown in the [accompanying] table.

Supplement per day	Total gain 3 week assay period—gm. Diet of Register et al.	Diet of Emerson
0	34	34
[illegible] γ B_{12}	[illegible]	[illegible]
[illegible] γ B_{12}	77	71
1 mg. *A*	48	34
5 mg. *A*	46 (1 dead)	
2 mg. *B*	34	[illegible]
5 mg. *B*	41	
2 mg. *C*	[illegible]	

Preliminary experiments [suggest] that *compound A* may be somewhat more effective when given orally, but no increase in effectiveness was noted for *compound B* when it was administered orally "

Hoffmann-LaRoche, Inc. Nutley N J

1119 BOXER, G E., RICKARDS, J C., ROSENBLUM, C., and WOODBURY, D T: *Vitamin B_{12} and cyanide metabolism*, Federation Proc. 10 166. March 1951

"Methods have been developed which permit the colorimetric determination and isolation, in a form suitable for isotope analysis, of millimicrogram quantities of

cyanide. The following cyanide fractions can be quantitatively separated from urine and tissue homogenates: 1) free (or loosely bound) cyanide, 2) cyanide liberated from vitamin B_{12} by illumination, 3) cyanide obtained by oxidation of thiocyanate (Boxer and Rickards, *Arch. Biochem.* In press) After injection of 500γ $C^{14}N$ (sp. act. 1 mc/mM) into a 9-kg. dog, about 30% of the radioactivity appeared in the urine over a period of 7 days. About half of this activity (15%) was present as thiocyanate representing the established detoxification mechanism for cyanide. The free (or loosely bound) cyanide excreted in the urine during the first 24 hours was of low specific activity indicating at least a 100-fold dilution in a rapidly turning over metabolic pool of cyanide. The cyanide bound in the vitamin B_{12} excreted in the first 24 hours had a specific activity about 12 times as high as the free cyanide and 4 times as high as the thiocyanate. This indication of a metabolic activity of the cyano group of B_{12}, independent from the rest of the molecule, was further emphasized by the fact that on the following day the specific activity of the B_{12} cyanide had fallen sharply apparently through interaction with the cyanide pool of low specific activity A small part of the activity in the urine was found in allantoin."

Merck & Co., Inc.
Rahway, N. J.

1120. STEKOL, J A., and WEISS S.: *Possible metabolic role of KCN in the rat as studied with C-14-KCN* Federation Proc. 10 252-253, March 1951.

"C-14-KCN was injected intraperitoneally into adult Wistar rats, and 24 hours later the radioactivity was determined in the whole blood, liver and carcass proteins, and in choline (isolated as the reineckate) and creatine (isolated as creatinine-potassium-picrate) All of these tissue components contained appreciable radioactivity Furthermore, C-14-KCN administered together with bromobenzene to rats was utilized for the synthesis of cysteine and acetic acid, as was revealed by the radioactivity located in the cysteine and in the acetic acid of the p-bromophenylmercapturic acid which was isolated from the urine. Aside from the well known metabolic conversion of cyanide to thiocyanate, these observations suggest possible formation and utilization of cyanide *in vivo* either per se or via formaldehyde-formate. Furthermore, the property of cyanides to reduce disulfides to the corresponding thionitriles, and the fact that vitamin B_{12} contains easily removable cyanide group, point to an interesting possibility of vitamin B_{12} as the carrier of the metabolically elaborated cyanide with its attendant hematopoietic and other physiological properties."

Lankenau Hospital Research Institute, and
Institute for Cancer Research
Philadelphia, Pa.

1121. PROCTOR, B. E., and LANG, D A. *Vitamin B_{12} content of soft and hard-shell clams,* Nature 168 36-37 July 7 1951 (Letter to Editor)

Fresh samples of soft and hard-shell clams contain more vitamin B_{12} in mcg./Gm. of dry weight, than pig, beef or calf liver The visceral mass of the clam contains a higher concentration of vitamin B_{12} than other parts. This material, which is discarded before clams are minced for canning, may prove to be an excellent source of vitamin B_{12} for animal feed supplements.

Massachusetts Institute of Technology
Cambridge, Mass.

1122. COATES M. E., FORD J E., HARRISON G. F., KON S K., PORTER, J W G., CUTHBERTSON W F J., and PEGLER, H. F *Vitamin B_{12} activity for chicks and different micro-organisms of gut contents and faeces* Biochem. J 49 lxvii, Oct. 1951 (in Soc. Proc.)

1123 SCHEID H. E., and SCHWEIGERT B. S. *Liberation and microbiological assay of vitamin B_{12} in animal tissues,* J Biol. Chem. 193 299-305 Nov 1951 (abstr J Am. Dietet. A. 28 156, Feb. 1952)

"A series of experiments was conducted with the use of various media and techniques to develop a more uniform assay for determining the vitamin B_{12} potency of animal tissue. Lactobacillus leichmannii 327 was used as the test organism. An amino acid medium that contained 2 mg. thioglycolic acid was found to be satisfactory and the results obtained were comparable to those obtained when aseptic additions or Seitz filtration techniques were used. The vitamin B_{12} potency of several meat samples was determined. Liver was shown to contain approximately twenty five times and kidney approximately ten to fifteen times as much vitamin B_{12} activity as pork, beef, or lamb muscle."

1124 FORD J E., KON S. K., and PORTER, J W G. *The multiple nature and potency for different micro-organisms of the vitamin B_{12} activity of calf rumen contents and faeces,* Biochem. J 50 ix, Dec. 1951 (in Soc. Proc.)

1125. FROST D V., FRICKE, H. H., and SPRUTH, H. C.: *Rat growth assay for vitamin B_{12}. Correspondence with collaborative microbiological assay results on U.S.P liver extracts,* Federation Proc. 11 443-444, March 1952.

"A rat growth assay for vitamin B_{12} using a highly purified diet with casein has been further studied and improved. Rats used in this study were from the laboratory colony These animals originally Wistar have been inbred over a period of 20 years. Animals purchased from 1 outside source responded satisfactorily while those purchased from 2 other suppliers did not. It is therefore clear that maternal diet is important in the preparation of weanling rats for this assay Two liver extract samples, used in 1950 U.S.P collaborative study of the microbiological vitamin B_{12} assay were assayed in rats at levels corresponding to average B_{12} values of 23 participating laboratories. The growth responses for the 2 liver extracts at 3 levels in the diet were in close agreement with the response to similar graded levels of crystalline vitamin B_{12}. The method was used to test the specificity of the microbiological method as a measure of the completeness of destruction of vitamin B_{12} in liver extract by treatment with sodium hydroxide. Results, which were in agreement between rat and microbiological assay indicated that destruction of vitamin B_{12} was not entirely complete even under conditions of drastic treatment.

antithyrotoxic factor of Ershoff does not appear to complicate this rat growth assay for vitamin B_{12}. Nicotinamide added to the diet depressed the growth of control and supplemented animals, but did not necessarily improve the sensitivity of the method."

Abbott Laboratories
North Chicago, Ill.

1126. DENTON C. A., KELLOGG W L., and BIRD H. R.: *The apparent vitamin B_{12} content of eggs as affected by extraction in the presence of cyanide*, Federation Proc. 11 440, March 1952.

"Vitamin B_{12} was estimated by the microbiological procedure set up by the U S. Pharmacopoeia. The vitamin was extracted by autoclaving the yolks (whites contain little or no B_{12}) with sodium acetate buffer at pH 4.5 for 30 minutes at 15 pounds, with and without sodium cyanide. *When cyanide was not used, yolks from hens* on an adequate diet gave an average value of .12 µg. of B_{12}/yolk (range .07 .15) With cyanide present, the value was .48 µg. (range .34-.56) When 1 µg. of B_{12} was added to each sample the average B_{12} value obtained was .84 µg./yolk, or a recovery of about 75% However when cyanide was present during the extraction, an average value of 1.4 µg. was obtained, which was a recovery of 95% In yolks from hens depleted of B_{12} the average value without cyanide was 0.2 µg./yolk (range 01 .027) With cyanide, the yolks assayed 11 µg of B_{12} (range .07 .18) When 1 µg. of B_{12} was added/yolk, without cyanide present, the average assay value was .81 µg. which represented a recovery of 79% With cyanide a value of 1.1 µg. was obtained which was about 99% recovery Similar effects with cyanide have been obtained with tissues and feces as well as with eggs. It appears that the usual methods for the extraction of B_{12} do not make available to the test organism all the added or naturally occurring B_{12} of eggs, tissues and feces."

U. S. Department of Agriculture
Beltsville, Md.

FOODSTUFFS AND FEEDSTUFFS

1127 PRODUCTS and PROCESSES *Assay of vitamin B_{12}*, Chem. & Engin. News 27 644 Feb. 28, 1949

"At Food Research Laboratories, Inc., work has been carried out on the assay of vitamin B_{12} in food and pharmaceutical preparations. The company is accepting samples for the microbiological assay of vitamin B_{12} activity as well as for the assay of animal protein factor activity by the chick growth method. Results of assays are expressed in terms of crystalline vitamin B_{12} or if desired, relative to known good sources of these factors, such as liver extracts and fish solubles. Methods are still under investigation for determining the best manner of releasing such bound or conjugated forms of vitamin B_{12} (or other substitute growth factors) "

1128. PENSACK, J M., BETHKE, R. M., and KENNARD D C. *Some properties of an unidentified growth factor present in fish products*, J Nutrition 37 353-360, March 1949.

Ohio Agricultural Experiment Station
Wooster Ohio

1129. RUEGAMER, W R.: *Occurrence of an unidentified rat growth factor in cottonseed meal*, Arch. Biochem. 23 236-238, Sept. 1949

Cottonseed meal contains a rat growth factor in contrast to soybean meal. It is not certain whether it is identical with vitamin B_{12} or whether it is some other antithyroid substance. Since considerable heat is applied in processing of the meal, the factor is relatively heat stable.

1130. LEWIS, U J., REGISTER, U D., THOMPSON, H. T., and ELVEHJEM, C. A.: *Distribution of vitamin B-12 in natural materials*, Proc. Soc. Exper Biol. & Med. 72 479-482, Nov 1949

Authors summary "The vitamin B-12 content of a number of natural materials as determined by the rat assay is presented. Fish solubles, streptomycin slops sheep rumen contents and glandular meats are excellent sources of vitamin B-12. Muscle tissue, eggs and milk products contain lesser amounts, whereas plant materials show no measurable activity "

1131. SCHEID H. E., and SCHWEIGERT B. S.: *Some factors affecting the potencies of vitamin B_{12} and Leuconostoc citrovorum factor of certain natural products*, J Biol. Chem. 185 1-8, July 1950.

Potencies of vitamin B_{12} and of the factor required by Leuconostoc citrovorum were determined in liver extracts, beef liver and beef round, and were found to be very similar when measured with Lactobacillus leichmannii 313 and 327 as test organisms. Potency of the Leuconostoc citrovorum factor and vitamin B_{12} varied independently The effect of autoclaving, hot and cold water extraction, alkali treatment, and enzymatic digestion on the potency of vitamin B_{12} and the citrovorum factor in liver extracts, beef liver and beef round is reported. These studies indicate that complete release of vitamin B_{12} from natural materials is difficult. Enzymatic technics or hot water extractions offer considerable promise as a means of releasing bound forms of the vitamin in natural materials.

American Meat Institute Foundation, and
University of Chicago
Chicago, Ill.

1132. BICKOFF E. M., LIVINGSTON A. L., and SNELL, N S. *The occurrence of vitamin B_{12} and other growth factors in alfalfa*, Arch. Biochem. 28 242 252, Sept. 1950.

Authors summary: "Microbiological assays for vitamin B_{12} in alfalfa using L. leichmannii as the test organism yielded total apparent vitamin B_{12} values ranging from 50 to 62 parts per billion (p.p.b) for fresh alfalfa and from 12 to 45 p.p.b. for dehydrated alfalfa meal. The values obtained varied with sample size, the smaller the sample, the higher the apparent B_{12} activity

"Employment of differential assay techniques such as alkali digestion to destroy B_{12}, inclusion of salt in the media which inhibits response to B_{12}, and paper-partition chromatography have indicated that more than 85% of the total apparent vitamin B_{12} activity is due to factors other than B_{12}. Chick assays indicate that the factors responsible for the apparent B_{12} activity in microbiological assays do not replace vitamin B_{12} for chick growth.

"Several techniques, including precipitation of impurities with acetone adsorption of activity on charcoal and chromatography on silica gel have proved successful in concentrating the factors responsible for the apparent B_{12} activity of alfalfa, but did not preferentially concentrate the pure B_{12}."

Western Regional Research Laboratory
Albany Calif.

1133. PEELER, H T., YACOWITZ, H., CARLSON C. W., MILLER, R. F., NORRIS L. C., and HEUSER G F: *Studies on the vitamin B_{12} content of feedstuffs and other materials* J Nutrition 43: 49-61 Jan. 1951

Authors summary "Evidence is presented that comparable values for vitamin B_{12} potency are obtained by chick growth and microbiological assays.

"Studies revealed that substances which replace vitamin B_{12} in the metabolism of L. leichmannii can be differentiated equally well with paper partition chromatography or by destruction of the vitamin with alkali.

"Using the rapid microbiological assay method and the alkali correction procedure, a study of the vitamin B_{12} content of feedstuffs and other materials was made. Fish meal and related products and liver products were found to contain appreciable quantities of vitamin B_{12}, while cereals and their by-products were found to contain very little, if any vitamin B_{12}.

"Two vitamin B_{12} activity peaks were obtained by paper partition of alfalfa meal extracts. The smaller faster moving peak appeared to be due to thymidine. The other slow moving peak may be due to a form of vitamin B_{12} not destroyed by alkali, as its R_f value did not correlate with that of any of the known active desoxyribosides."

Cornell University
Ithaca, N Y

1134 TARR, H L. A. *Microbiological formation of vitamin B_{12} in fishery waste materials* Federation Proc. 10 257 March 1951.

"Since vitamin B_{12} has been produced microbiologically largely by commercial concerns little information has been made available regarding substrates employed or yields obtained. Though this vitamin occurs abundantly in most fish tissues, especially in the viscera, its distribution in these and in by products such as fish meals and condensed solubles is very variable (Tarr *et al. Food Technology* 4 354, 1950) With a view to increasing, and possibly standardizing, the vitamin B_{12} content of such products *Streptomyces griseus* and *S aureofaciens* have been cultured with simple aeration in herring press water adjusted to 2% solids content and with no additive other than 2 µg/gm. of cobalt. Microbiological determination of vitamin B_{12} in the crude fermentation liquids indicated that maximum yields were obtained after about 4 days at 27° No agreement whatever was obtained in the results of assays of the crude fermentation products with *Lactobacillus leichmannii* in tube turbidimetric procedure and with a cup-plate assay employing a glucose-inorganic salt medium inoculated with a vitamin B_{12} requiring mutant of *Escherichia coli.* In general the vitamin B_{12} content as indicated by *L. leichmannii* assay (about 400 mµg/ml) was somewhat similar to that obtained following chromatographic separation on KH_2PO_4 impregnated filter paper development on agar inoculated with the *E coli* mutant, and subsequent assay of the eluted vitamin. It appears that the hydroxy cobalamine form of vitamin B_{12} occurs generally in these fermentation products. On treatment with KCN (2.5 mg/ml.) at pH 5.5 about one-half the total activity of the crude products appears as vitamin B_{12} following chromatographic separation. All activity disappears on treatment with 0.2 N NaOH at 100 for 30 minutes."

Pacific Fisheries Experimental Station
Vancouver B C.

Index of Subjects

ABSTRACT

ABSTRACT

ABSTRACT

ABSTRACT

ABSTRACT

ABSTRACT

Abstract

Index of Authors

D

I

J

K

ABSTRACT

L

M

ABSTRACT

N

O

P